

ACTA UNIVERSITATIS SZEGEDIENSIS

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# ACTA BIOLOGICA

NOVA SERIES

TOMUS XLI

FASCICULI 1-4

SZEGED (HUNGARIA)  
1995/1996

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Adjuvantibus  
**A. BARANYI, L. GALLÉ, K. GÜLYA, E. MIHALIK, J. NEMCSÓK,  
S. TÓTH, F. ZSOLDOS**

redigit  
**GYULA L. FARKAS**

editionem curat  
**GYÖRGY GYÖRFFY**

edit  
Facultas Scientiarum Naturalium Universitatis Szegediensis de Attila József nominatae

Nota  
Acta Biol. Szeged.

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A szerkesztő bizottság tagjai  
**BARANYI ATTILA, GALLÉ LÁSZLÓ, GÜLYA KÁROLY,  
MIHALIK ERZSÉBET, NEMCSÓK JÁNOS, TÓTH SÁNDOR, ZSOLDOS  
FERENC**

Szerkesztő  
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Technikai szerkesztő  
**GYÖRFFY GYÖRGY**

Kiadja  
a Szegedi József Attila Tudományegyetem Természettudományi Kara  
(Szeged, Aradi vértanúk tere 1.)

ISSN 0324-6523  
ISSN 0563-0592

Kiadványunk rövidítése  
Acta Biol. Szeged.



**IN MEMORIAM DR. HABIL. SÁNDOR GULYÁS**  
**(1933-1996)**



DR. SÁNDOR GULYÁS, retired head of the Department of Botany and director of the Botanical Garden, has died at the age of 63.

He was born on April 9, 1933 in Köröstarcsa. From 1952 he attended the József Attila University and graduated in 1956 as a biology-chemistry teacher. He joined the Department of Botany in 1960 as an Assistant Lecturer and remained there continuously onwards. He became a Lecturer in 1963 and a Reader in 1970. He received his "Dr. Univ." degree in 1962 and became a "Candidate of the science of biology" in 1971.

He was involved in lecturing and leading laboratory practicals in Plant Morphology and Anatomy. His special course "Flower biology" always attracted many interested students, and formed a real contact between his academic activities and bee-keeping, his favourite pastime which he performed with proficiency. He was a beloved leader of students preparing their master's theses, and gave a helping hand to numerous students starting their scientific work as student scientists. 14 of his students were awarded first prizes at National Conferences of Student Scientists, for which he was given the title of "Master of Students' Leaders" by the Ministry of Education and Culture. He twice received the award "For Outstanding Work".

He was a real social man. He was a member of the Botanical Committee of the Hungarian Academy of Sciences, president of the Plant Morphology and Anatomy Working Committee, editor of the scientific journal "Botanikai Közlemények", and co-editor of "Acta Biologica Szegediensis", and for a shorter period "Tiscia" and "Méhészet".

He was Deputy Dean of the Faculty of Science between 1980 and 1983, and a member of the University Council from 1983 to 1990.

He was involved in supporting the study and scientific work of talented students. He established a foundation for the "Improvement of Plant Anatomy", and was a member of the presidium of the "Frank-Helianthus" and "VARGA BÉLÁNÉ" foundations. As a consultant of numerous Ph.D. students, he contributed significantly to the formation of a new generation in the science of botany.

He published 147 scientific papers, besides books and a university textbook.

He was head of the Department of Botany and director of the Botanical Garden from 1982. During this period, he contributed to the significant enlarging of the existing collection of living plants at the Botanical Garden. With his well-balanced, cheerful personality, he created a calm and constructive atmosphere at his department until he retired on July 1, 1996. Unfortunately, he could not enjoy his long-awaited retirement, as he was attacked by a rapid and fatal illness.

He is remembered by hundreds of biology and biology-teacher students and numerous botanist colleagues nationwide. His memory will continue to live on with honour and sincere appreciation.

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## FACTORS INFLUENCING THE IMMOBILIZATION OF GLUCOAMYLASE

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(Received: March 19, 1996)

### Abstract

Glucoamylase produced by *Aspergillus niger* was covalently attached to a polyacrylamide bead support possessing carboxylic functional groups activated by water-soluble carbodiimides. Factors influencing the immobilization were studied. The most favourable carbodiimide for the immobilization was N-t-butyl-N'-dimethylaminopropyl carbodiimide methyl iodide. In the experiments in which N-cyclohexyl-N'-morpholinoethyl carbodiimide methyl tosylate was used as coupling agent, the optimum medium was 0.1 M potassium phosphate buffer (pH 7.5). The support can be saturated with protein. In accordance with the molecular weight of the glucoamylase, supports with an exclusion limit of 100,000 daltons or more proved to be most advantageous.

**Key words:** Glucoamylase, *Aspergillus niger*, immobilization, polyacrilamide support, covalent bonds, carbodiimide effects, medium, support porosity.

### Introduction

Starch is very important industrial raw material. Amylolytic enzymes play an indispensable role in its processing.  $\alpha$ -Amylase can only be employed in soluble form, since the molecular weight of its substrates amylose and amylopectin are too high for satisfactory hydrolysis with immobilized enzymes. The second enzyme involved in the saccharification of starch is glucoamylase (1,4- $\alpha$ -D-glucan glucohydrolase, EC 3.2.1.3.).

Innumerable attempts have been made to immobilize this enzyme (cf. HARTMEIER, 1988). We have found that glucoamylase can be immobilized effectively by covalent attachment to a synteic polycarboxylic matrix activated by awater-soluble carbodiimide (SZAJÁNI et al., 1985). In connection with continuous ethanol production through use of a coupled immobilized enzyme-immobilized cell reactor system, the factors influencing the immobilization of glucoamylase were studied in detail.



## Materials and Methods

### Materials

Glucoamylase with a specific activity of 900-1500 units/g protein was produced by *Aspergillus niger*. Akrilex C, polyacrylamide bead polymers containing carboxylic functional groups were commercial products of Reanal. Carbodiimides were synthesized according to JÁSZAY et al. (1987). Soluble starch was a preparation of E. Merck GmbH Co. (Darmstadt, Germany). All other chemicals were reagent grade commercial preparations of Reanal (Budapest, Hungary).

### General methods of immobilization

Glucoamylase was covalently attached to Akrilex C bead polymers possessing carboxylic functional groups activated by a water-soluble carbodiimide, described earlier (SZAJÁNI et al., 1985). The general method of immobilization was as follows:

Akrilex C xerogel (1 g) was suspended and swollen in 50 ml of potassium phosphate buffer. The water-soluble carbodiimide, in a stoichiometric quantity relativ to the carboxylic functional groups located on the support, dissolved in 25 ml of cold (0 °C) buffer, was added with continuous stirring and cooling in an ice-bath. After 10 min, 25 ml of enzyme solution was added, and the pH was adjusted to the starting pH value. The mixture was incubated at 0-4 °C for 48 h, with two 6-h periods of agitation. The gel was filtered off by suction and successively washed three times with 100 ml of buffer, three times with 100 ml of buffer containing 1.0 M sodium chloride, three more times with 100 ml of buffer to remove unbound proteins, and finally, with a large volume of distilled water to remove the buffer ions. The products were lyophilized.

### Measurement of protein

Protein determination were performed according to the method of LOWRY et al. (1951) as modified by SCHARTERLE and POLLACK (1973). The amount of immobilized protein was calculated from the difference between the amount of protein introduced into the reaction mixture and the protein present in the filtrate and washing solutions after immobilization.

### Assay of glucoamylase activity

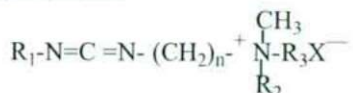
The activities of both soluble and immobilized glucoamylases were determined by measuring the amount of D-glucose liberated from soluble starch. The method routinely used was based on the iodometric titration of D-glucose (ERDEY, 1956).

In the activity test of the soluble enzyme, the reaction mixture (5.1 ml) contained 40 mg/ml soluble starch (pH 4.0) and 5-12 µg/ml enzyme. After an appropriate incubation time (30-90 min) at 60 °C, the reaction was terminated by alkali treatment. The control containing only substrate was treated in an identical manner. In the case of immobilized glucoamylase, 1.5-2.0 mg of immobilized enzyme suspended in 5.0 ml of 40 mg/ml soluble starch (pH 3.8) was stirred for an appropriate time (45-120 min) at 60 °C. The enzyme was then filtered off quickly (a few seconds) and the concentration of liberated D-glucose was determined. One unit is defined as the amount of enzyme which catalyses the liberation of one gram of D-glucose from soluble starch per hour at pH 4.0 (soluble enzyme) or pH 3.8 (immobilized enzyme) at 60 °C.

## Results and discussion

### *Effect of carbodiimide structure on immobilization of glucoamylase*

On a theoretical basis, it was supposed that disubstituted carbodiimides characterized by the general formula





could effect the immobilization process. Therefore, over 30 carbodiimides bearing different substituents were synthesized and screened for enzyme immobilization. The catalytic activities of the immobilized enzymes were influenced advantageously by the structure of the carbodiimide used as coupling agent (SZAJÁNI et al., 1991). Data concerning the immobilization of glucoamylase are listed in Table 1. For the highest catalytic activity of the immobilized enzyme, the most favourable carbodiimide structures were those in which  $R_1$  = tert-butyl;  $R_2$  = methyl;  $R_3$  = methyl ;  $n = 3$ ; and  $X$  = iodide or 4-methyl-toluene sulphonate.

In the further experiments, commercially available N-cyclohexyl-N'-morpholinoethyl carbodiimide methyl tosylate was used as coupling agent.

#### *Effect of pH of coupling reaction mixture*

In an earlier experiment (SZAJÁNI et al., 1985) in 0.1 M potassium phosphate at 0 °C, in which N-cyclohexyl-N'-morpholinoethyl carbodiimide methyl tosylate was used, it was found that the optimum pH for the coupling is 7.5.

#### *Effect of ionic strength of coupling reaction mixture*

The effect of the ionic strength of the coupling reaction mixture was studied in potassium phosphate solution (pH 7.5) at 0-4 °C, N-cyclohexyl-N'-morpholinoethyl carbodiimide methyl tosylate being used as coupling agent (Table 2). The ionic strength dependence shows an apparent maximum. It is presumed that the function reflects a complex phenomenon involving changes in pH, ionization, hydration and diffusion resistances.

#### *Effect of protein concentration of coupling reaction mixture*

The effect of the protein concentration of the coupling reaction mixture was studied in 0.1 M potassium phosphate (pH 7.5) at 0-4 °C, with N-cyclohexyl-N'-morpholinoethyl carbodiimide methyl tosylate as coupling agent (Fig. 1). The support can be saturated with protein.

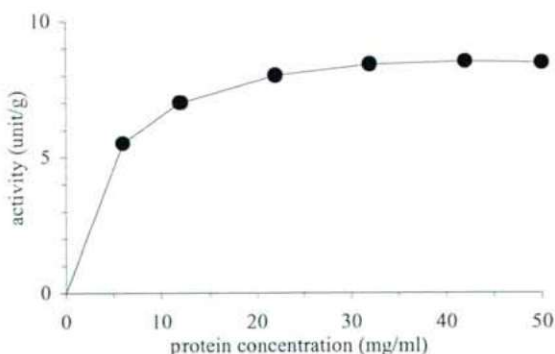

















Fig. 1. Effect of protein concentration of coupling reaction mixture. Experiments were performed in 0.1 M potassium phosphate buffer (pH 7.5) at 0 °C. Akrilex C-100 xerogel (200 mg) was activated with N-cyclohexyl-N'-morpholinoethyl carbodiimide methyl tosylate (400 mg), glucoamylase was then added.

Table 1. Effect of carbodiimide structure on immobilization of glucoamylase

Carbodiimide structure					A	B	C	D
R <sub>1</sub>	R <sub>2</sub>	R <sub>3</sub>	n	x <sup>-</sup>				
CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	2	I <sup>-</sup>	0	0	0	0
CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	3	I <sup>-</sup>	3.2	1.4	0.3	93.7
CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	3	H <sub>3</sub> C-SO <sub>3</sub> <sup>-</sup>	3.8	7.2	0.4	95.6
CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	3	Cl <sup>-</sup>	0	0	0	0
CH <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	3	Br <sup>-</sup>	1.8	96	0.2	95.8
CH <sub>3</sub>	CH <sub>3</sub> -CH <sub>2</sub>	CH <sub>3</sub>	3	I <sup>-</sup>	0	0	0	9
CH <sub>3</sub> -CH <sub>2</sub>	CH <sub>3</sub>	CH <sub>3</sub>	2	I <sup>-</sup>	16.3	2.6	1.3	0
CH <sub>3</sub> -CH <sub>2</sub>	CH <sub>3</sub>	CH <sub>3</sub>	3	Cl <sup>-</sup>	13.5	13	1	21.1
CH <sub>3</sub> -CH <sub>2</sub>	CH <sub>3</sub>	CH <sub>3</sub>	3	H <sub>3</sub> C-SO <sub>3</sub> <sup>-</sup>	15.4	2.3	1	0
CH <sub>3</sub> -(CH <sub>2</sub> ) <sub>2</sub>	CH <sub>3</sub>	CH <sub>3</sub>	2	I <sup>-</sup>	0	0	0	0
CH <sub>3</sub> -(CH <sub>2</sub> ) <sub>2</sub>	CH <sub>3</sub>	CH <sub>3</sub>	2	H <sub>3</sub> C-O-SO <sub>3</sub> <sup>-</sup>	8.6	19.1	2.6	15.7
CH <sub>3</sub> -(CH <sub>2</sub> ) <sub>2</sub>	CH <sub>3</sub>	CH <sub>3</sub>	2	Br <sup>-</sup>	0	0	0	0
CH <sub>3</sub> -(CH <sub>2</sub> ) <sub>2</sub>	CH <sub>3</sub>	CH <sub>3</sub>	3	I <sup>-</sup>	0	0	0	0
CH <sub>3</sub> -(CH <sub>2</sub> ) <sub>2</sub>	CH <sub>3</sub>	CH <sub>3</sub>	3	H <sub>3</sub> C-SO <sub>3</sub> <sup>-</sup>	4.5	3	1	0
CH <sub>3</sub> -(CH <sub>2</sub> ) <sub>2</sub>	CH <sub>3</sub>	CH <sub>3</sub>	3	Br <sup>-</sup>	0	0	0	0
CH <sub>3</sub> -(CH <sub>2</sub> ) <sub>2</sub>	CH <sub>3</sub>	CH <sub>3</sub>	3	I <sup>-</sup>	17.3	82.2	3.3	1.9
CH <sub>3</sub> -(CH <sub>2</sub> ) <sub>2</sub>	CH <sub>3</sub>	CH <sub>3</sub>	3	H <sub>3</sub> C-SO <sub>3</sub> <sup>-</sup>	13.7	3	1.9	9.1
CH <sub>3</sub> -(CH <sub>2</sub> ) <sub>3</sub>	CH <sub>3</sub>	CH <sub>3</sub>	3	Br <sup>-</sup>	10	11.5	3	20.1
	CH <sub>3</sub>	CH <sub>3</sub>	3	I <sup>-</sup>	41.6	10	3.8	5.7
	CH <sub>3</sub>	CH <sub>3</sub>	3	H <sub>3</sub> C-SO <sub>3</sub> <sup>-</sup>	37.8	5	4.2	2.2
	CH <sub>3</sub>	CH <sub>3</sub>	2	I <sup>-</sup>	17.8	7.4	3.8	26.3
	CH <sub>3</sub>	CH <sub>3</sub>	2	H <sub>3</sub> C-SO <sub>3</sub> <sup>-</sup>	8.9	13.5	2.7	30.3
	CH <sub>3</sub>	CH <sub>3</sub>	3	I <sup>-</sup>	11	12	5	11.8
	CH <sub>3</sub>	CH <sub>3</sub>	3	H <sub>3</sub> C-SO <sub>3</sub> <sup>-</sup>	9	-	2.8	0
	CH <sub>3</sub>	CH <sub>3</sub>	3	Br <sup>-</sup>	15.8	2.6	1.5	31.8
	CH <sub>3</sub>	CH <sub>3</sub>	3	Br <sup>-</sup>	1.8	-	0.2	95.8
	CH <sub>3</sub>	CH <sub>3</sub>	2	I <sup>-</sup>	16.3	6.1	1.7	32.7
	CH <sub>3</sub>	CH <sub>3</sub>	2	H <sub>3</sub> C-SO <sub>3</sub> <sup>-</sup>	17.7	5	2.2	7.8
	CH <sub>3</sub>	CH <sub>3</sub>	3	I <sup>-</sup>	22.3	8.3	2.5	9.9
	CH <sub>3</sub>	CH <sub>3</sub>	3	I <sup>-</sup>	17.3	4	1.2	0
	CH <sub>3</sub>	CH <sub>3</sub>	3	H <sub>3</sub> C-SO <sub>3</sub> <sup>-</sup>	17.3	8.3	2.3	0
	CH <sub>3</sub>	CH <sub>3</sub>	3	I <sup>-</sup>	0.5	0.5	0.1	98
	CH <sub>3</sub>	CH <sub>3</sub>	3	Br <sup>-</sup>	17.3	18.7	1.4	47.2

A: Activity on dry wt basis (units/g solid); B: Activity on protein basis (%)<sup>a</sup>; C: Activity bound (%)<sup>b</sup>; D: Activity loss (%)<sup>b</sup>

<sup>a</sup> The activity of the soluble enzyme was taken as 100%.

<sup>b</sup> The total activity introduced into the coupling reaction mixture was taken as 100%.

*Effect of porosity support on immobilization of glucoamylase*

In a study of the effect of the support porosity on the immobilization of glucoamylase, a series of Akrix C bead polymers characterized by the molecular exclusion limit were used.

Experiments were performed in 0.1 M potassium phosphate buffer (pH 7.5) at 0-4 °C, with N-cyclohexyl-N'-morpholinoethyl carbodiimide methyl tosylate as coupling agent (Table 3). The results were in good agreement with the data concerning the molecular weight of *Aspergillus niger* glucoamylase (FOGARTY and BENSON, 1983).

Table 2. Effect of ionic strength of coupling reaction mixture on immobilization of glucoamylase<sup>a</sup>.

Ionic strength	Activity (units g <sup>-1</sup> solid)
0.127	1.3
0.052	1.9
0.134	5.8
0.268	10.9
0.536	4.2

<sup>a</sup>Experiments were performed in potassium phosphate buffer (pH 7.5) at 0-4 °C. Akrix C-100 xerogel (200 mg) was activated with N-cyclohexyl-N'-morpholinoethyl carbodiimide methyl tosylate (400 mg), and glucoamylase (250 mg) was then added.

Table 3. Effect of porosity of support on immobilization of glucoamylase<sup>a</sup>.

Support	Exclusion limit (dalton)	Activity (units g <sup>-1</sup> solid)
Akrix C-30	30,000	5.1
C-60	60,000	5.8
C-100	100,000	9.5
C-200	200,000	9.7

<sup>a</sup>Experiments were performed in 0.1 M potassium phosphate buffer (pH 7.5) at 0-4 °C. Akrix C xerogel (200 mg) was activated with N-cyclohexyl-N'-morpholinoethyl carbodiimide methyl tosylate (400 mg), and glucoamylase (250 mg) was then added.

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## STUDY OF THE OPERATION OF CO-IMMOBILIZED GLUCOSE-6-PHOSPHATE ISOMERASE AND GLUCOSE-6-PHOSPHATE DEHYDROGENASE IN A FLOW INJECTION SYSTEM

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(Received: November 18, 1996)

### Abstract

Glucose-6-phosphate isomerase and glucose-6-phosphate dehydrogenase were co-immobilized covalently on Akrilex C 100, a polyacrylamide bead polymer possessing carboxylic functional groups, using a water-soluble carbodiimide for activation of the support. The activities of the immobilized isomerase and dehydrogenase were 110.8 U/g solid and 48.4 U/g solid, respectively.

The immobilized enzymes were packed in a reactor (3 ml), the enzyme reactor was inserted into the flow injection system, and the operation was studied with glucose-6-phosphate and fructose-6-phosphate as substrates. A linear relationship was observed between the substrate concentration and the peak in the concentration range 0.2-1 mM for both substrates. The dependence of the peak area on the sample volume and the flow rate was also linear. The immobilized enzymes exhibited good operational stability during operation for more than 5 months. In a coupled system with hexokinase, the applicability of the co-immobilized enzymes for the determination of fructose and glucose in different wines and fruit juices was demonstrated.

*Key words:* co-immobilized glucose-6-phosphate isomerase and dehydrogenase, flow injection analysis, co-determination of fructose and glucose

### Introduction

Enzymatic analysis is highly sensitive and specific and is of increasing importance in practical applications. If the enzymes are immobilized, they can be repeatedly employed for many analyses (LOWE, 1985; HO, 1988). Much attention has recently been devoted to the determination of biologically important substances and food components through the use of biospecific sensors or analysers. Flow methods such as flow injection analysis can readily be automated and they have therefore become important (RUZICKA and HANSEN, 1981; LUNDBACK and OLSSON, 1985).

Enzymes attached covalently to a polyacrylamide support containing carboxylic functional groups have been found to have advantageous properties for both preparative processes and analytical measurements (SZAJÁNI et al., 1980; KOTORMAN et al., 1991; SIMON et al., 1992).

Glucose-6-phosphate dehydrogenase and glucose-6-phosphate isomerase are widely used in clinical chemistry and food analysis for the enzymatic determination of NADP<sup>+</sup> and hexose phosphates, and for the determination of enzyme activities (phosphoglucumutase and hexokinase). Glucose-6-phosphate dehydrogenase was previously immobilized for NADPH production. (SIMON et al., 1994). The present paper reports on the co-immobilization of glucose-6-phosphate dehydrogenase with glucose-6-phosphate isomerase. The operation of these enzymes in a flow injection system was studied.

## Materials and Methods

### Materials

Glucose-6-phosphate dehydrogenase (D-glucose-6-phosphate: NADP<sup>+</sup> 1-oxidoreductase, EC 1.1.1.49) was isolated from bakers' yeast according to NOLTMAN et al. (1961). The specific activity of the enzyme was 2.6 U/mg protein. Glucose-6-phosphate isomerase (D-glucose-6-phosphate ketol-isomerase, EC 5.3.1.9) was isolated from rabbit muscle (Noltman, 1966). The specific activity of the enzyme was 110 U/mg protein. Akrix C 100, a polyacrylamide bead (100-320 µm) polymer containing carboxylic functional groups (6.4 mequiv./g xerogel) was a commercial product of Reanal (Budapest, Hungary). 1-Cyclohexyl-3-(2-morpholinoethyl)carbodiimide metho-4-toluenesulfonate was purchased from Fluka (Buchs, Switzerland). All other chemicals were commercial, reagent grade preparations (Reanal).

### Immobilization

The covalent attachment of glucose-6-phosphate dehydrogenase and glucose-6-phosphate isomerase to Akrix C was carried out according to SZAJANI et al. (1980).

### Measurement of protein

Protein determination was performed as described by LOWRY et al. (1951). The amount of immobilized protein was determined indirectly from the difference between the amount of protein introduced into the coupling reaction mixture and the amount of protein present in the filtrate and washing solutions after immobilization.

### Assay of enzyme activities

The glucose-6-phosphate dehydrogenase activity was measured in 0.1 M triethanolamine buffer (pH 7.6) containing 1.6 mM glucose-6-phosphate, 0.37 mM NADP<sup>+</sup>, 6.7 mM MgCl<sub>2</sub> and 0.3 U/ml glucose-6-phosphate dehydrogenase. The reaction was initiated by the addition of enzyme. The rate of reaction was calculated from the increase in absorbance at 340 nm at 25°C. The activity of the immobilized enzyme was measured in the same reaction mixture, as follows: 100 mg of immobilized enzyme was suspended and swollen in 5 ml reaction mixture. The suspension was continuously stirred with a mechanical stirrer for an appropriate time (usually 1-10 min) at 25°C. The immobilized enzyme was then filtered off quickly and the amount of NADPH was determined at 340 nm. One unit of enzyme activity (U) was defined as the amount of enzyme that catalyses the formation of 1 µmol of NADPH per minute at 25°C.

The activities of the soluble and immobilized glucose-6-phosphate isomerase were determined by measuring the rate of transformation of glucose-6-phosphate to fructose-6-phosphate as described by ROE (1934). One unit of enzyme activity was defined as the amount of enzyme which catalyses the formation of 1 µmol of fructose-6-phosphate per minute at 30°C.

### Analytical system

In the analytical measurements, LKB 2238 Uvicord S II equipment (Broma, Sweden) with a flow-through cell (10 µl) was used to monitor the changes in absorbance at 365 nm. The output signal was displayed on an OH-814/1 recorder (Radelkis, Budapest, Hungary). The carrier stream was pumped with an LKB 2132 micropex peristaltic pump.

### *Wine and juice analysis*

The samples were diluted 50-700-fold to obtain appropriate glucose/fructose concentrations for measurement. A 2 ml aliquot was taken, containing 2.7 mM ATP, 6.7 mM  $\text{MgCl}_2$  and 10 U hexokinase was added. After standing for 20 min at room temperature, the samples were diluted 1:1 with 10 mg/ml  $\text{NADP}^+$  and analysed in a flow injection system.

## **Results**

For the covalent immobilization of glucose-6-phosphate dehydrogenase and isomerase enzymes Akrilex C 100 support was used. The enzymes were co-immobilized on the same support under optimized conditions. The catalytic activity of the immobilized glucose phosphate isomerase was 110.8 U/g xerogel, and that of the dehydrogenase was 48.4 U/g xerogel. A 3 ml bed reactor (isomerase activity: 3.5 U, dehydrogenase activity: 2.1 U) was introduced into a flow injection system. The carrier stream was composed of 100 mM triethanolamine buffer, pH 8.0, 5 mM  $\text{MgCl}_2$  and 2 mM  $\text{NADP}^+$ . The glucose-6-phosphate and fructose-6-phosphate concentrations varied in the range 0.2-1 mM. Conditions affecting the operation of this two-enzyme system were studied. The peak shape (A) and the dependence of the peak area on the glucose-6-phosphate concentration (B) are shown in Fig. 1. Up to 1 mM substrate concentration, a linear relationship was observed. Similar results were obtained with fructose-6-phosphate as substrate (Fig. 2). The operation of the two enzymes in co-immobilized form did not need a much longer reaction time than the one-enzyme system. The peak shapes for injection of different volumes of glucose-6-phosphate (A) and fructose-6-phosphate (B) are presented in Fig. 3. The reaction times for measurement in the one- and the two-enzyme systems were the same. The changes in peak shape at different flow rates for the one- (A) and two-enzyme (B) systems are shown in Fig. 4. The best and reproducible results were obtained at the higher flow rates of 80-100 ml/h.

Calibration curves with NADPH standard at different flow rates are shown in Fig. 5. In the concentration range 0.2-1 mM, linear calibration curves were obtained.

During these experiments the operational stability of the enzyme reactor for both enzyme activities was tested (Fig. 6). The enzyme reactor was stable and no measurable decrease in activity could be observed after operation for 5 months. The enzyme reactor was stored at 8 °C in a refrigerator after measurements.

The practical application of this two-enzyme reactor for the determination of glucose and fructose in Hungarian wines and fruit juices was studied. The very low levels of sugar phosphates in wines and juices was under the detection limit of our system, and therefore in a coupled reaction with soluble hexokinase the glucose and fructose amounts were determined. The approach used in this work was based on the following reaction sequences:



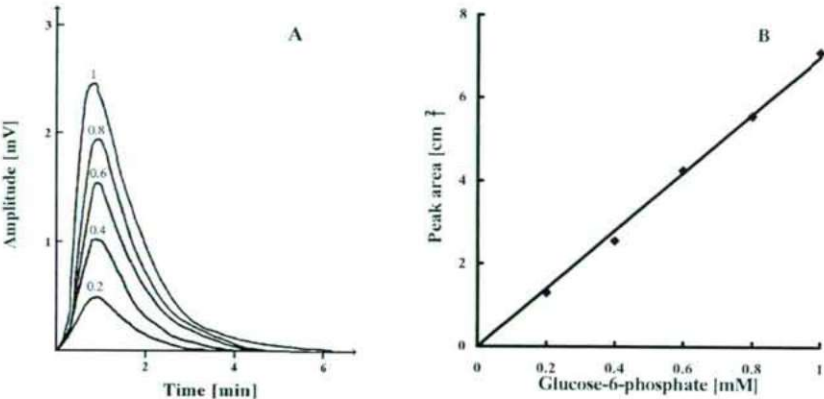
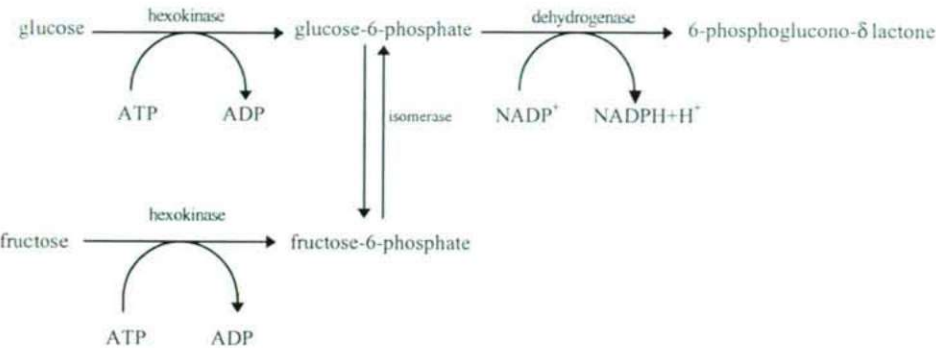


Fig. 1. Peak shapes (A) and change in peak area with glucose-6-phosphate concentration (B).  
Flow rate: 100 ml/h. Sample volume: 100  $\mu$ l.

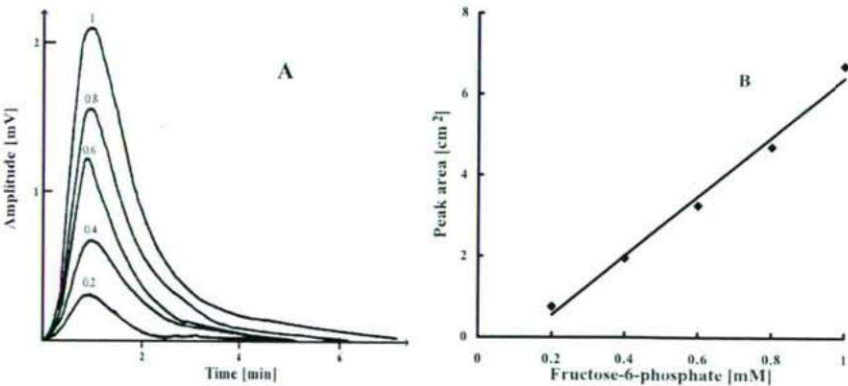


Fig. 2. Peak shapes (A) and change in peak area with fructose-6-phosphate concentration (B).  
Flow rate: 100 ml/h. Sample volume: 100  $\mu$ l.



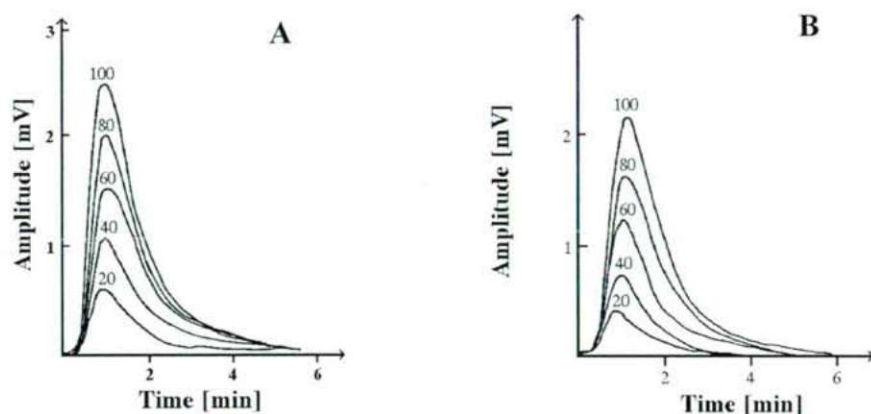


Fig. 3. Responses on injection of 20, 40, 60, 80 and 100 µl. 1 mM glucose-6-phosphate (A), 1 mM fructose-6-phosphate (B). Flow rate: 100 ml/h.

The results summarized in Table 1 are the mean values of 10 measurements. Determinations in the flow injection system were compared with traditional soluble enzyme measurements. The data agreed within a 5% error and suggest the applicability of this system for practical purposes.

Table 1. Co-determination of glucose and fructose in wines and fruit juices with soluble hexokinase and immobilized glucose-6-phosphate dehydrogenase/ isomerase in a flow injection system. The flow rate was 100 ml/h, with 100 µl of sample. (The results are the mean values of 10 measurements.)

	Glucose and fructose (g / l)	
	with soluble enzymes	with immobilized enzymes
Wine brands		
Zengő	14.26	14.07
3 puttonyos tokaji aszu	196.20	187.28
Édes szamorodni	103.44	99.09
Furmint	13.87	13.87
Fruit juices		
Apple	59.25	58.86
Apricot	75.90	76.49
Grape	114.94	112.96

## Discussion

Co-immobilized glucose-6-phosphate dehydrogenase and isomerase were prepared on Akrilex C 100, a polyacrylamide bead support. The immobilized catalytic activity was 110.8 U/g for the isomerase and 48.4 U/g for the dehydrogenase.

The operation of the immobilized enzymes in a flow injection system was studied. A linear response was obtained with glucose-6-phosphate and fructose-6-phosphate in

the concentration range 0.2-1 mM. The shapes of the peaks varied with the flow rate of the carrier stream and the sample volume. At a flow rate of 100 ml/h, using 100  $\mu$ l of sample, the flow injection system operates with the same reaction time for both substrates and the results are reproducible.

The practical application of the two-enzyme reactor in flow injection analysis was investigated. The comparative co-determination of the glucose and fructose contents in wines and fruit juices offers a simple and novel method for routine analysis with the accuracy of classical soluble enzyme measurements. The enzyme reactor displayed good operational stability: an activity decrease was not observed during 5 months of operation.

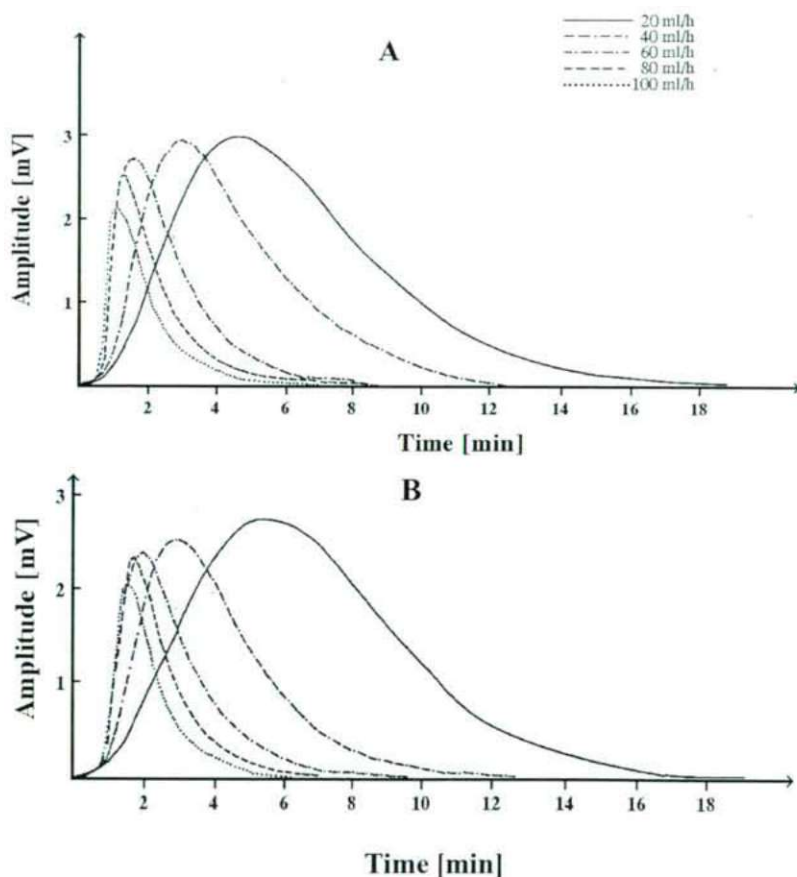


Fig. 4. Responses with flow rates of 20, 40, 60, 80 and 100 ml/h. (A) with glucose-6-phosphate, (B) with fructose-6-phosphate as standards.

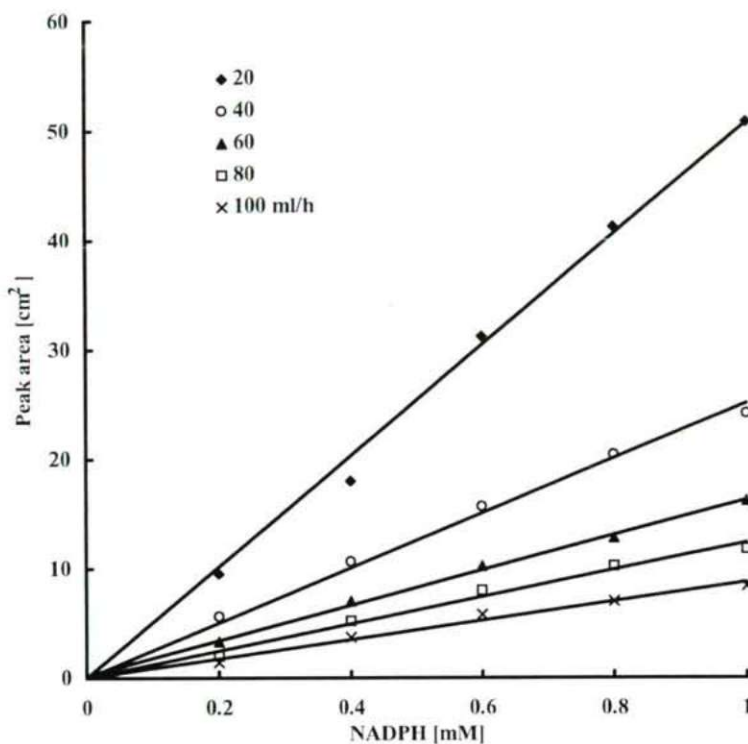


Fig. 5. Peak area as a function of NADPH concentration at different flow rates. Sample volume: 100  $\mu$ l.

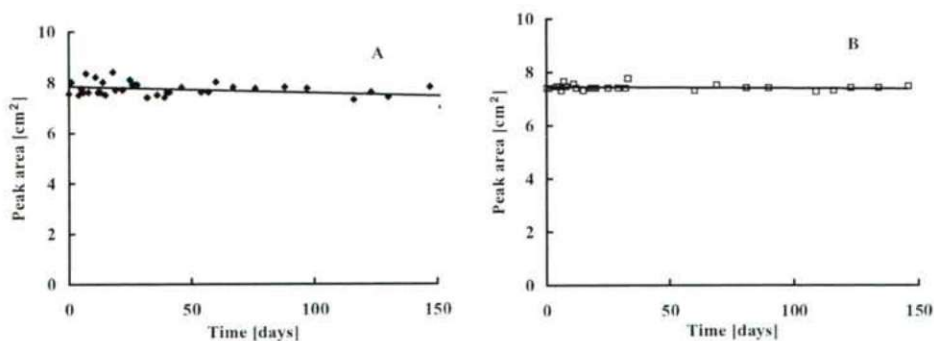


Fig. 6. Operational stability of co-immobilized glucose-6-phosphate dehydrogenase and isomerase bioreactor. Glucose-6-phosphate dehydrogenase activity (A) and isomerase activity (B) with 1 mM glucose-6-phosphate (fructose-6-phosphate) standard solution.

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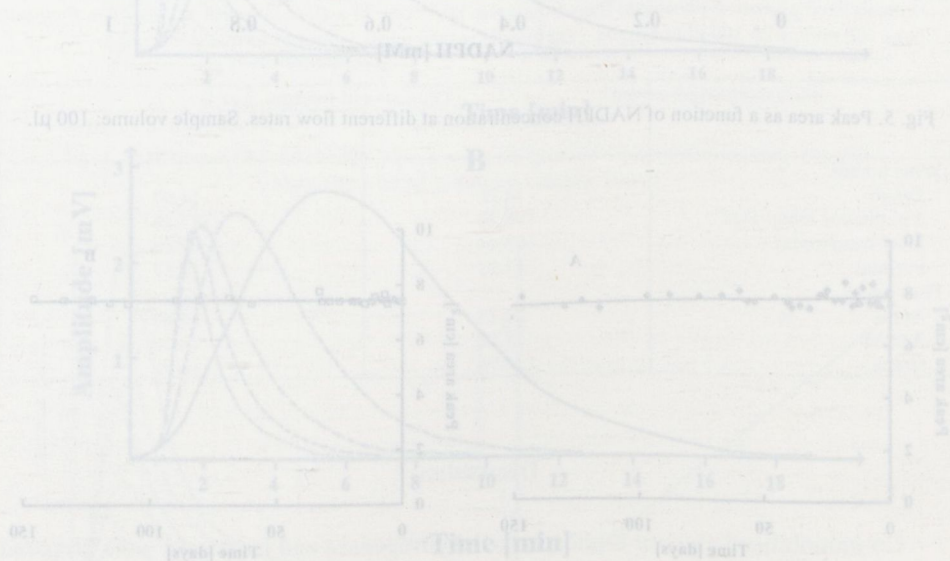


Fig. 6. Operational stability of co-immobilized glucose-6-phosphate dehydrogenase and isomerase. Glucose-6-phosphate dehydrogenase activity (A) and isomerase activity (B) with 1 mM glucose-6-phosphate (fructose-6-phosphate) standard solution.



# AGE AND PALAEOENVIRONMENT OF THE SPHERULITE-BEARING POLÁNY MARL FORMATION (UPPER CRETACEOUS, HUNGARY) ON BASIS OF PALYNOLOGICAL AND NANNOPLANKTON INVESTIGATION

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(Received: January 15, 1996)

## Abstract

Spherulites and spherulite like forms microlapilli found in formations of different ages may be indicators of geological events in extraterrestrial space and faraway areas of the Earth. The age and conditions of embedding can be deciphered by investigating the enclosing rocks. Studying the Upper Cretaceous in this way may be of special importance in tracing the latest Cretaceous extinction as well.

The borehole Nagygörbő-1 can be considered as the "Spherulite-reference section" in the Upper Cretaceous Polány Marl Formation, because the spherulites described by VASKÓ-DÁVID (1994) occurred in these biostratigraphically well determined host sediments (SIEGL-FARKAS and WAGREICH, 1994).

*Key words:* Campanian, correlation, palynology, dinoflagellata, nannoplankton, palaeoenvironment.

## Introduction

In Hungary, Upper Cretaceous deposits can be found in four facies realms. So far spherulites are known only from the Transdanubian Central Range area. There the

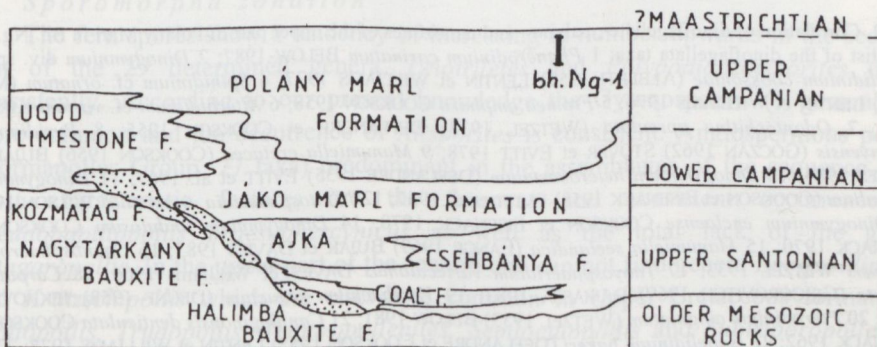


Fig. 1. Simplified section to illustrate the relationships of Senonian formations in the Transdanubian Central Range with position of bh. Ng-1.

whole Senonian sequence lies with the fluvial-lacustrine Csehbánya Formation or the palustrine Ajka Coal Formation over the older Mesozoic rocks. These are followed by different marine formations, the youngest of them being the Polány Marl Formation (Fig. 1).

Till now, spherulite occurrences have been established in the Csehbánya, Ajka Coal and Polány Marl Formations.

The borehole Nagyörbő-1 (Keszthely Mt.) penetrated the upper section of the Polány Marl between 1332.8-1515.0 m. The biostratigraphic study of the section and the correlation of the biozones allow a more accurate dating of the formation.

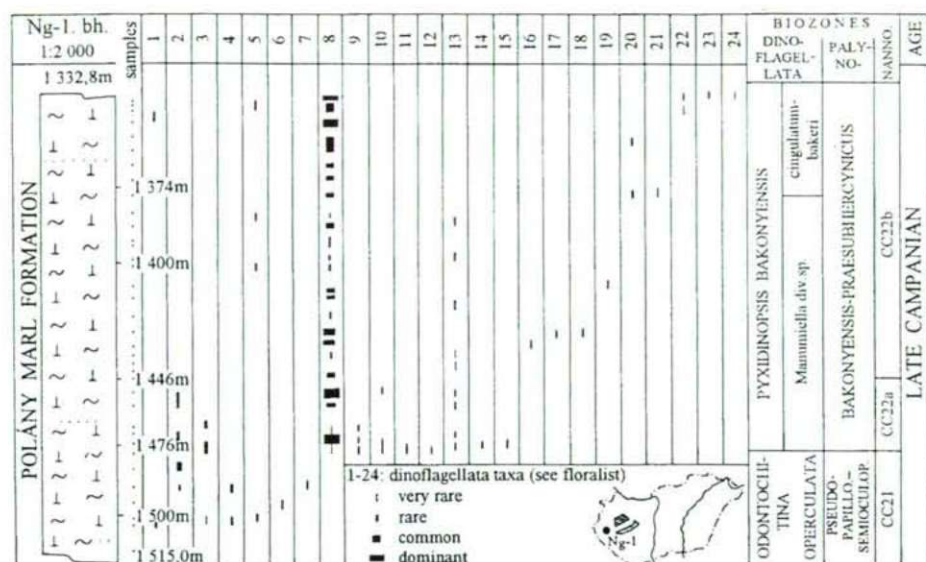


Fig. 2. Correlation of dinoflagellate-, palyno- and nanozones of the upper part of Polány Marl in Bh. Ng. 1. Floralist of the dinoflagellate taxa: 1 *Phanerodinium carinatum* BELOW 1987; 2 *Dinogymnium* div. sp.; 3 *Isabelidinium cooksoniae* (ALBERTI 1959) LENTIN et WILLIAMS 1977; 4 *Spinidinium* cf. *ornatum* (MAY 1980) LENTIN et WILLIAMS 1976; 5 *Fromea amphora* COOKSON 1958; 6 *Alterbidinium* cf. *varium* KIRSCH 1991; 7 *Odontochitina operculata* (WETZEL 1933) DEFLANDRE et COOKSON 1955; 8 *Pyxidinospis bakonyensis* (GÓCZÁN 1962) STOVER et EVITT 1978; 9 *Manumiella cretacea* (COOKSON 1956) BUIÁK et DAVIES 1983; 10 *Dinogymnium heterocostatum* (DEFLANDRE 1935) EVITT et al. 1967; 11 *Dinogymnium westralium* (COOKSON et EISENACK 1958) EVITT et al. 1967; 12 *Chitosphaeridia everricula* WILSON 1974; 13 *Dinogymnium euclaense* COOKSON et EISENACK 1970; 14 *Dinogymnium undulosum* COOKSON et EISENACK 1970; 15 *Manumiella seelandica* (LANGE 1969) BUIÁK et DAVIES 1983; 16 *Cannosphaeropsis utinensis* WETZEL 1933; 17 *Tanyosphaeridium variecalamus* DAVEY et WILLIAMS 1966; 18 *Carpatella cornuta* (GRIGOROVITCH 1969) DAMASSA 1983; 19 *Veryhachium reductum* (DEUNF 1958) JEKHOVSKY 1961; 20 *Pterodinium cingulatum* (WETZEL 1933) BELOW 1981; 21 *Canninginopsis denticulata* COOKSON et EISENACK 1962; 22 *Isabelidinium bakeri* (DEFLANDRE et COOKSON 1955) LENTIN et WILLIAMS 1977; 23 cf. *Pareodinia aphelia* COOKSON et EISENACK 1958; 24 *Paleocystodinium* sp.



### Palynological investigations

Both rich terrigenous spore-pollen and marine phytoplankton assemblages could be studied. The individual biozones and their correlation can be seen in Fig. 2, while the most significant palynomorphs in Plate I-III.

#### *Dinoflagellata zonation*

24 taxa could be determined. The dinoflagellates appear at 1503.0 m and from there downwards they occur consistently. Two assemblage zones and inside the youngest, two subzones could be designated. (SIEGL-FARKAS and WAGREICH, 1996; SIEGL-FARKAS 1996)

#### *Odontochitina operculata* Assemblage Zone (1467.0-1515.0 m)

The scattered occurrence of dinoflagellates is characteristic. The eponyms of the zone and the *Spinidinium ornatum* as well as the *Alterbidinium varium* occur only here, each being represented only by a single specimen. The *Dinogymnium* div. sp. occurs more frequently.

#### *Pyxidinospis bakonyensis* Assemblage Zone (1332.8-1476.0 m)

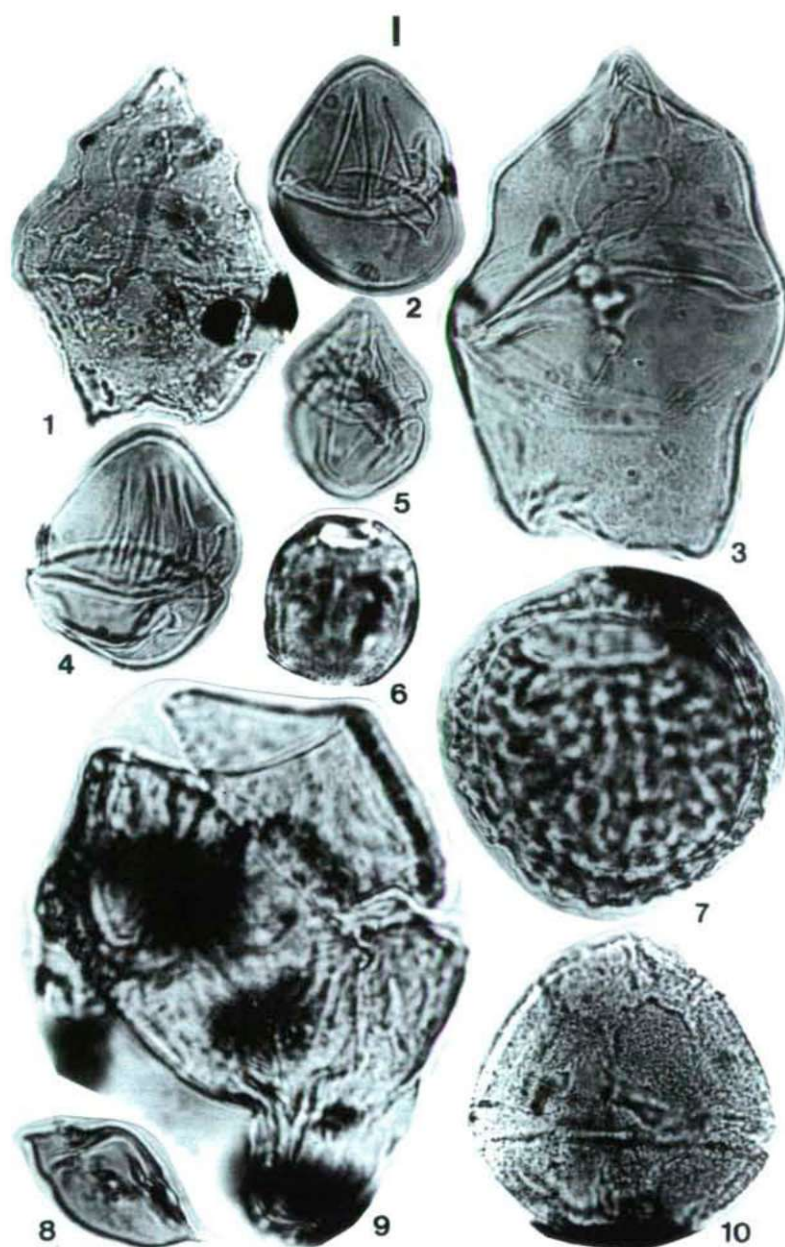
The *Pyxidinospis bakonyensis* occurs frequently and consistently. On the basis of the dinoflagellates appearing and disappearing beside it, the zone can be divided into two subzones.

*Manumiella* div. sp. Subzone (1380.0-1476.0 m): *Manumiella cretacea*, *Manumiella seelandica*, *Isabelidinium cooksoniae*, *Cannosphaeropsis utinensis*, *Dinogymnium* div. sp. are present only a few samples, beside the consistent occurrence of *Dinogymnium euclaense*. Apart from these, other determined species can be found only in a few samples and are represented by a few specimens only.

*Pterodinium cingulatum*-*Isabelidinium bakeri* Subzone (1332.8-1380.0 m): the assemblage dominated by *Pyxidinospis bakonyensis* is completed by *Pterodinium cingulatum*, *Canninginopsis denticulata*, *Pareodinia aphelia* and *Isabelidinium bakeri*, which have been identified only here.

#### *Sporomorpha zonation*

The fern spores show a tendency of increasing in number upwards in the section. Out of the 29 determined genera, *Leiotriletes* and *Polypodiaceoisporites* occur consistently. According to our present knowledge the Gymnosperms (5 genera) are more common and the occurrence of *Alisporites* is consistent. Angiospermous pollen (Normapolles Group, 27 taxa) predominant in the assemblages of the sequence. The most characteristic features are the frequent and consistent occurrence of *Pseudopapillopollis praesubhercynicus* as well as the total lack of the genus *Hungaropollis*. In the upper part of the section one can not find genera *Longanulipollis* and *Krutzschipollis* which are elsewhere common in Upper Cretaceous formations. *Oculopollis*, *Trudopollis*, *Triporopollenites*, *Semioculopollis* and *Subtriporopollenites* occur consistently but in small number.





On the basis of the presence of the determined genera *Concavipollis*, *Interpollis*, *Labrapollis*, *Nudopollis* and *Plicapollis* a similarity can be noticed with the assemblages known in the southern part of the Great Hungarian Plain (SIEGL-FARKAS, 1986).

Taking into account the above listed facts, two zones can be designated: the *Pseudopapillopollis-Semioculopollis* Assemblage Zone (1476.0-1515.0 m), and above it the *Palaeostomocystis bakonyensis-Pseudopapillopollis praesubhercynicus* Assemblage Zone.

### Redeposition

In the course of the investigations some Carboniferous (*Tripartites* sp.) and Upper Triassic (*Classopollis* sp., *Corollina* sp.) sporomorphs were determined.

### Animal remnants

In the sequence, organic Foraminifer tests and *Scolecodonta* (Annelidae) remnants occur consistently.

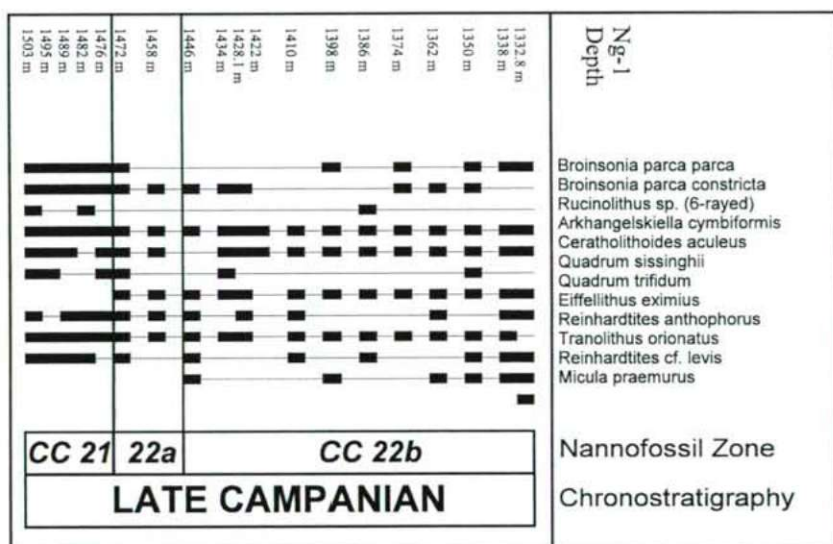
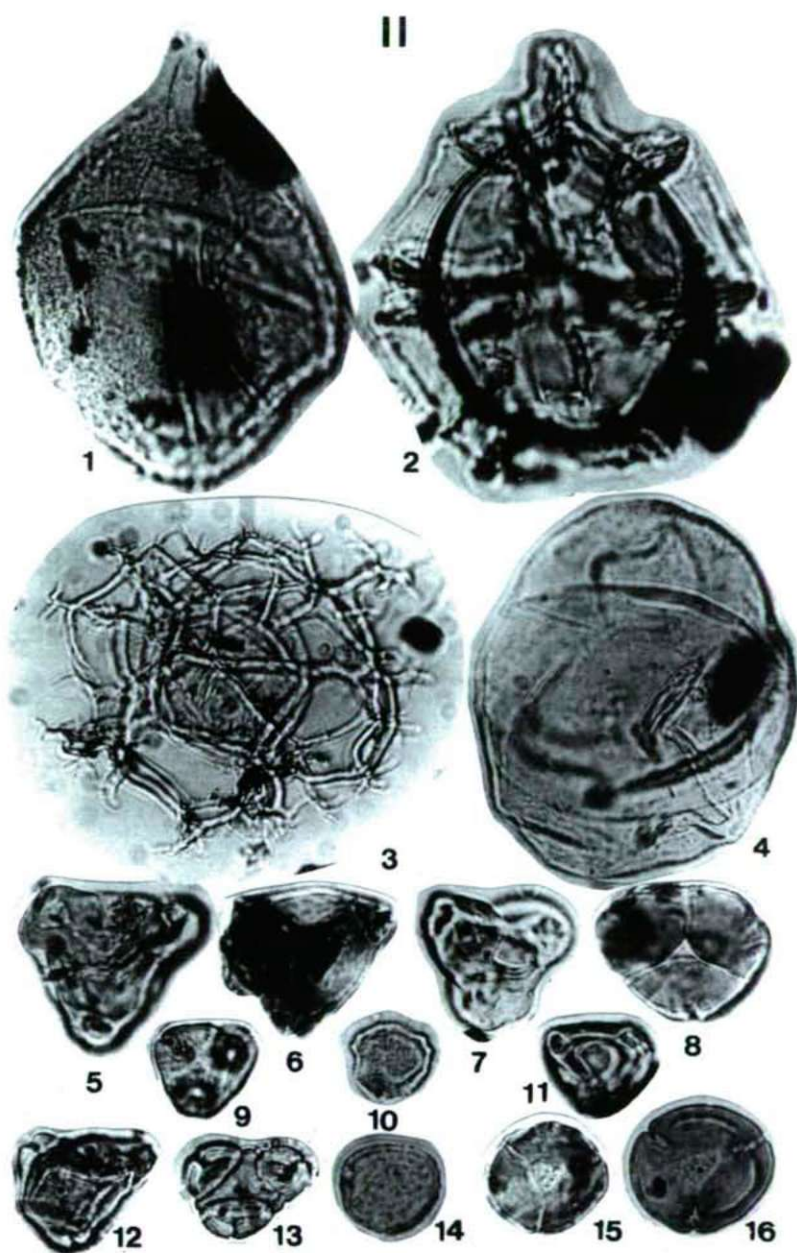


Fig. 3. Distribution of calcareous nannofossil marker species in the investigated Ng-1 section, 1332.8-1503.0 m.

Plate I. 1 *Spinidinium ornatum* (May) BUJAK et DAVIES (1489.0 m); 2 *Dinogymnium euclaense* COOKSON et EISENACK (1452.0 m); 3 *Isabelidinium cooksoniae* (ALBERTI) LENTIN et WILLIAMS (1503.0 m); 4 *Dinogymnium euclaense* COOKSON et EISENACK (1440.0 m); 5 *Dinogymnium euclaense* COOKSON et EISENACK (1458.0 m); 6 *Fromea amphora* COOKSON (1404.0 m); 7 *Pyxidinospis bakonyensis* (GÓCZÁN) STOVER et EVITT (1472.0 m); 8 *Verhachium irregulare* JEKHOVSKY (1410.0 m); 9 cf. *Odontochitina operculata* (WETZEL) DEFLANDRE et COOKSON (1489.0 m); 10 *Canninginopsis denticulata* COOKSON et EISENACK (1368.0 m).



*Calcareous Nannofossils*

19 samples of the Ng-1 core interval from 1332.8 m to 1503.0 m were investigated for their calcareous nannofossil content. Smear slides of the samples were analysed under the light microscope. The preservation and abundance of the nannofossils was generally moderate, although some samples contained well preserved nannofossils (Table 1).

Two nannofossil zones, one of them divided into 2 subzones according to the standard zonation of the Cretaceous of SISSINGH (1977) and PERCH-NIELSEN (1985) could be distinguished (Fig. 3, Plate IV).

*Quadrum sissinghii* Zone (CC21) (Authors: SISSINGH, 1977; PERCH-NIELSEN, 1985), (1503.0-1472.0m)

The marker species *Quadrum sissinghii* occurs together with other typical nannofossils of the Campanian, e. g. *Broinsonia (Aspidolithus) parca constricta*, *Broinsonia (Aspidolithus) parca parca*, *Ceratolithoides aculeus*, *Arkhangelskiella cymbiformis* and *Reinhardtites anthophorus*. The nannofossil zone CC21 can be correlated with the middle part of the Late Campanian (PERCH-NIELSEN, 1985; SCHÖNFELD and BURNETT, 1991).

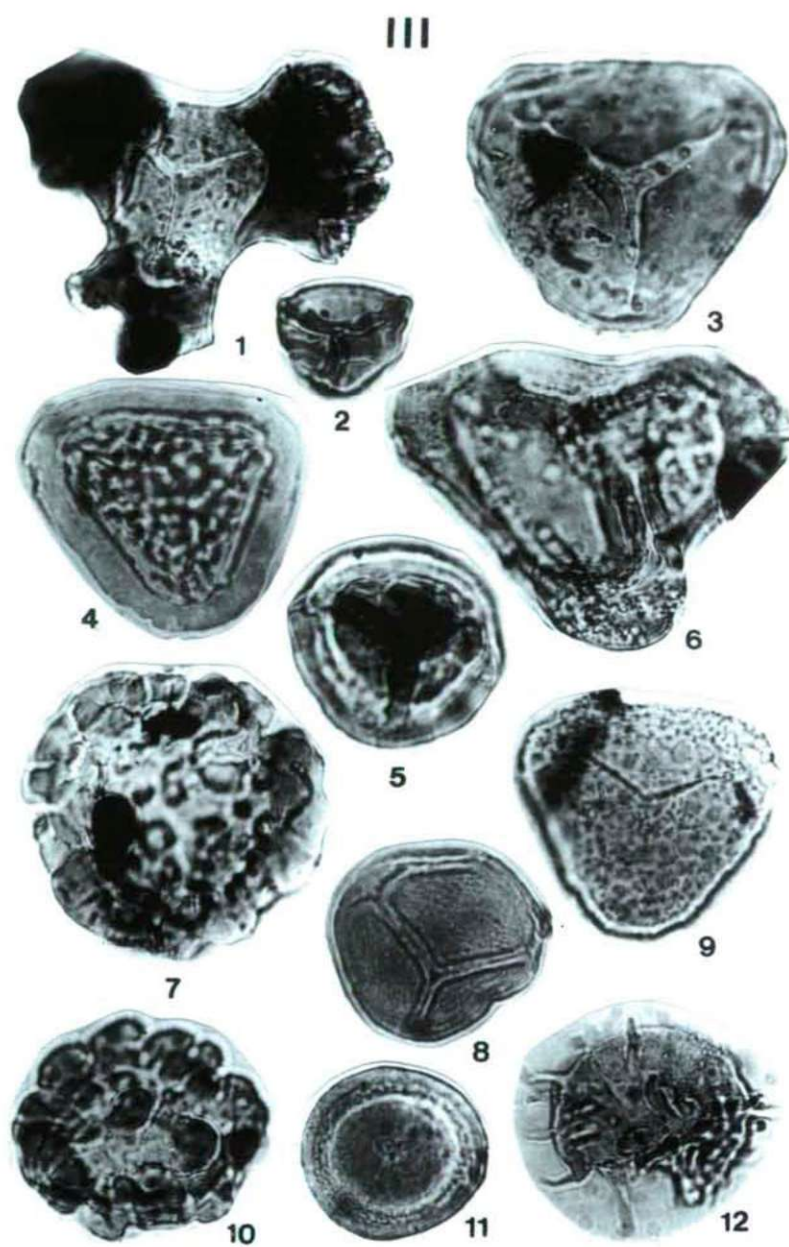
*Quadrum trifidum* Zone (CC22) (Authors: SISSINGH, 1977; PERCH-NIELSEN, 1985), (1472.0-1332.8 m)

CC22a The lowermost subzone of the *Quadrum trifidum* Zone ranges from 1472 m to 1458.0 m. It is defined by presence of *Quadrum trifidum* without *Reinhardtites levis*, although transitional forms from *Reinhardtites anthophorus* to *R. levis* (SISSINGH, 1977) are already present. Subzone CC22b starts in 1446.0 m and goes up to 1332.8 m. A 1446.0 m large *Reinhardtites* with a completely closed central area occur for the first time. They are grouped into *R. cf. levis* (SISSINGH, 1977; WAGREICH and KRENMAYR, 1993).

According to the correlations of SCHÖNFELD and BURNETT (1991) and WAGREICH and KRENMAYR (1993), the upper boundary of the Campanian defined by the last occurrence of the planktonic Foraminifer *Globotruncanita calcarata* falls at the base or into the nannofossil zone CC22c. Therefore the investigated Ng-1 core section from 1503.0 m to 1332.8 m is of middle to late Late Campanian age in the sense of Tethyan zonations.

Plate II. 1 *Pareodinia aphelia* COOKSON et EISENACK (1386.0 m); 2 *Pterodinium cingulatum* (WETZEL) BELOW (1344.0 m); 3 *Cannosphaeropsis utinensis* WETZEL (1434.0 m); 4 *Manumiella cretacea* (Cookson) BUJAK et DAVIES (1476.0 m); 5 *Interporopollenites nennhausensis* KRUTZSCH (1332.8 m); 6 *Placopollis pseudoexcellus* GREIFELD (1468.0 m); 7 *Interporopollenites gracilis* GÖCZÁN et SIEGL-FARKAS (1468.0 m); 8 *Oculopollis* sp. (1410.0 m); 9 *Semioculopollis minimus* GÖCZÁN (1344.0 m); 10 *Labrapollis labraferus* (POTONIE) KRUTZSCH (1452.0 m); 11 *Interpollis cf. microsupplingensis* KRUTZSCH (1392.0 m); 12 *Triatriopollenites lubomirovae* (GLADKOVA) KEDVES (1350.0 m); 13 *Suemegipollis germanicus* KRUTZSCH (1503.0 m); 14 *Pseudopapilopollis praesubhercynicus* GÖCZÁN (minor typ) (1332.0 m); 15 *Pseudopapilopollis praesubhercynicus* GÖCZÁN (1452.0 m).





## Age and correlation possibilities

### *Antecedents*

The palynostratigraphic standard zonation of GÓCZÁN (1964) divides the formations of the Bakony Mts. into eight (A-H) zones and gives the time of their deposition as Upper Santonian-Upper Maastrichtian. In this work of 1973 he assigned the whole of the Polány Marl Formation to the Maastrichtian and provided the zones with the names of the characteristic sporomorphs (GÓCZÁN, 1973). He assigned the Lower Maastrichtian formations to the *Pseudopapillopollis-Semioculopollis minimus* Assemblage Zone, while giving Dinoflagellata and Normapollis taxa names to the section representing the Upper Maastrichtian: *Palaeostomocystis bakonyensis-Pseudopapillopollis praesubhercynicus* Assemblage Zone.

SIEGL-FARKAS (1986) correlated the Senonian formations of borehole Bácsalmás-1 in the southern Great Hungarian Plain with the formations of the Bakony Mts. assigned to the Maastrichtian and distinguished two subzones in the Lower Maastrichtian: *devecserensis* and *sahi*.

The later correlation works (GÓCZÁN and SIEGL-FARKAS 1989, 1990; SIEGL-FARKAS 1993a) used the same stratigraphic subdivision.

The Foraminifera zonation of the formations of the Bakony Mts. designated the Campanian-Maastrichtian boundary in the lower third of the Polány Marl Formation (SIDÓ in GÓCZÁN 1973). The mollusc examinations gave a stratigraphic subdivision similar to the palynozones (CZABALAY in GÓCZÁN 1973).

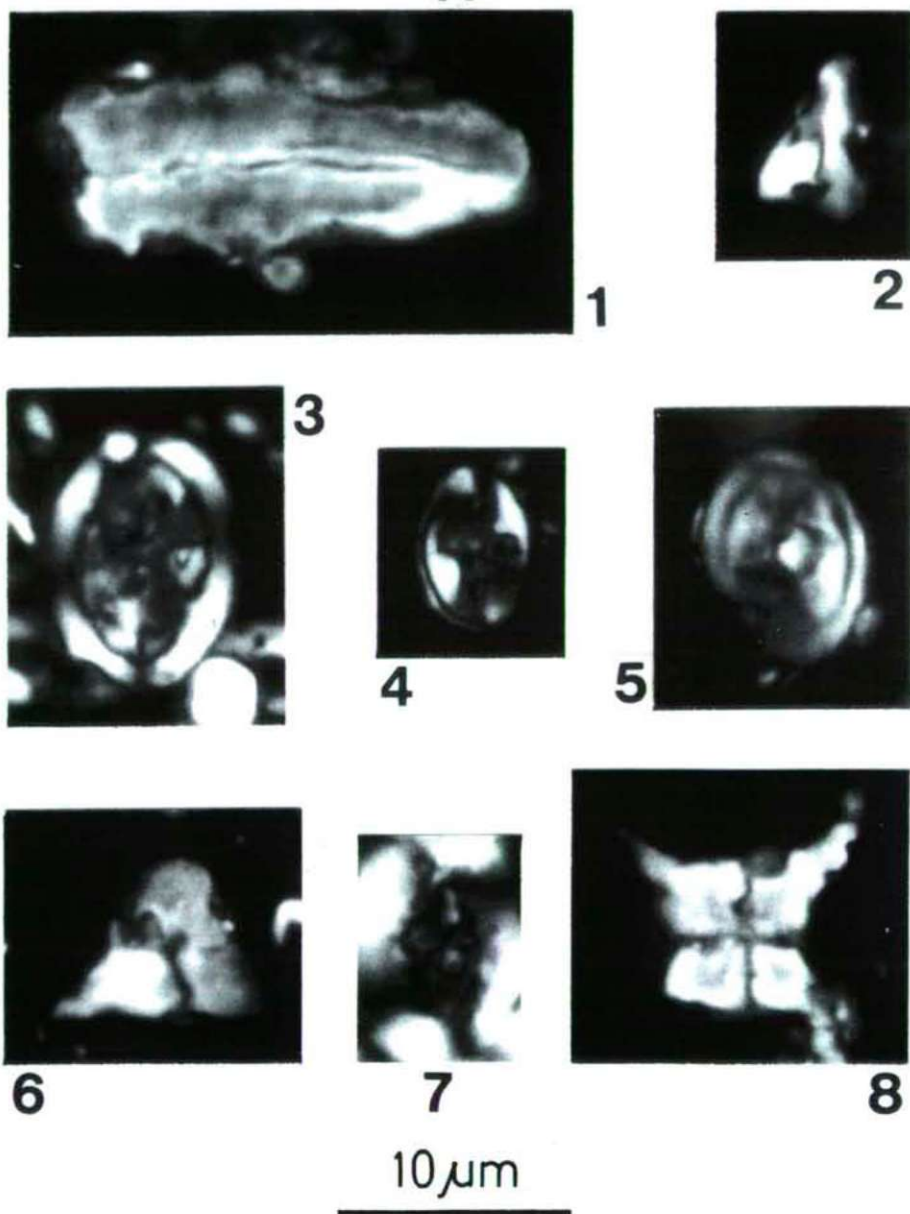
### Recent correlation results

For correlating the biostratigraphic standard zonation established in 1964 to the chronostratigraphic scale, we used the Dinoflagellata and Nannoplankton biostratigraphic data based on systematic investigations (Fig. 2).

On the basis of the so called global dinoflagellata zonation of WILLIAMS and BUJAK (1985), it would be reasonable to draw the Campanian-Maastrichtian boundary where *Odontochitina operculata* is replaced by *Dinogymnium euclaense* (1476.0 m). (This would be coincide with the Lower and Upper Maastrichtian respected of GÓCZÁN.) This is, however, hampered by the fact that the mentioned literature does not contain sufficient Tethyan data. Thus even the comparison of the dinoflagellata

Plate III. 1 *Tripartites* sp. (1422.0 m); 2 *Undulatisporites* sp. (1344.0 m); 3 *Bikolisporites* sp. (1374.0 m); 4 *Polypodiaceoisporites* cf. *granulatus* KEDVES (1458.0 m); 5 *Tauocusporites* sp. (1404.0 m); 6 *Matonisporites* cf. *weylandi* DÖRING (1422.0 m); 7 *Uvaesporites neerlandicus* HERNGREEN et al. (1434.0 m); 8 *Todisporites* sp. (1434.0 m); 9 *Vadaszporites* cf. *urkuticus* DEÁK (1356.0 m); 10 *Uvaesporites neerlandicus* HERNGREEN et al. (1434.0 m); 11 *Corollina* sp. (1392.0 m); 12 *Echinatisporites* sp. (1476.0 m). Magnification is 1000 ×, except Pl. II. fig. 3: 500 ×.

## IV





associations of the Boreal and Mediterranean regions is still problematic. Since *Dinogymnium euclaense* occurs only in the middle section (1380.0-1476.0 m) of borehole Nagyörbő-1 it can be regarded only as of local value in the currently elaborated zonation.

According to the data of WILLIAMS and BUJAK (1985), the first occurrence of *Cannosphaeropsis utinensis* at 1434.0 m would suggest still Late Santonian age.

This dinoflagellata zonation covering the upper formations of the Transdanubian Central Range shows a lot of uncertainties due to the lack of literary data. A better chronostratigraphic framework is provided by Nannoplankton correlation. On the basis of the Nannofossils, the sections between 1472.0-1515.0 m could be assigned to the CC21, between 1458.0-1472.0 m to the CC22a and between 1332.8-1446.0 to the CC22b Nannofossil Zones.

The dinoflagellata zonation established on the basis of the investigation of the sequence correlates surprisingly well with the Nannofossil zonation elaborated for the Upper Cretaceous Gosau Group of the Northern Calcareous Alps (WAGREICH and KRENMAYR, 1983).

The *Odontochitina operculata* Assemblage Zone designated between 1476.0-1515.0 m correlates well to the CC21 Nannofossil Zone and the *Pyxidinopsis bakonyensis* Assemblage Zone between 1332.8-1476.0 m corresponds to the CC22ab Nannofossil Zones.

For the Tethyan realm the upper boundary of the Campanian is drawn at the extinction level of the planktonic foraminifera *Globotruncanita calcarata* (e. g. BIRKELUND et al., 1984; SCHÖNFELD and BURNETT 1991). Detailed investigations of the Campanian-Maastrichtian boundary by SCHÖNFELD and BURNETT (1991) and KENNEDY et al. (1992) showed a diachronism between the current Tethyan and Boreal definitions of this stage boundary by correlations of various fossil groups. The last occurrence of *G. calcarata* (upper boundary of the Campanian) in the Tethyan realm falls into the nannofossil zone CC22 (CC22c after SCHÖNFELD and BURNETT 1991, top of CC22ab after WAGREICH and KRENMAYR 1993) whereas according to the Boreal definition (base on *Belemnella lanceolata* Zone) the boundary is in the nannofossil zone CC23a (SCHÖNFELD and BURNETT, 1991). The last nannofossil event recognised in borehole Ng-1, the first occurrence of *Reinhardtites cf. levis*, defines a level well below both the Tethyan and the Boreal definition of the Maastrichtian. Therefore the deposition of the Polány Marl Formation in the studied borehole took place during the Late Campanian.

In Hungary, younger Upper Cretaceous formations can be expected to all probability in the vicinity of Ganna, in the northern part of the Bakony Mts.

Plate IV. 1 *Lucianorhabdus cayeuxii* DEFLANDRE (1374.0 m); 2 *Ceratolithoides aculeus* (STRADNER) PRINS et SISSINGH (1374.0 m); 3 *Arkhangelskiella cymbiformis* VEKSHINA (1428.1 m); 4 *Eiffelithus eximius* (STOVER) PERCH-NIELSEN (1374.0 m); 5 *Reinhardtites levis* PRINS et SISSINGH (1428.1 m); 6 *Quadrum trifidum* (STRADNER in STRADNER et PAPP) PRINS et PERCH-NIELSEN in MANIVIT et al. (1428.1 m); 7 *Broinsonia (Aspidolithus) parca parca* (STRADNER) BUKRY (1374.0 m); 8 *Quadrum siissinghii* PERCH-NIELSEN (1428.1 m). All figures with crossed nicols.

On the basis of the recent investigations the deposition of the Transdanubian Upper Cretaceous marine formation (Jákó Marl) started in the late stage of the Late Santonian (SIEGL-FARKAS, 1993b; LANTOS et al., 1996; SIEGL-FARKAS and WAGREICH, 1996) and the youngest marine formation (Polány Marl) was deposited at the end of the Late Campanian.

Table 1. Alphabetic list of Calcareous nannofossil species

- Ahmuellerella octoradiata* (GORKA 1957) REINHARDT 1964  
*Arkhangelskiella cymbiformis* VEKSHINA 1959  
*Biscutum constans* (GORKA 1957) BLACK 1959  
*Biscutum* sp.  
*Braarudosphaera bigelowi* (GRAN et BRAARUD 1935) DEFLANDRE 1959  
*Broinsonia (Aspidolithus) parca constricta* HATTNER, WIND et WISE 1980  
*Broinsonia (Aspidolithus) parca parca* (STRADNER 1963) BUKRY 1969  
*Calculites obscurus* (DEFLANDRE 1959) PRINS et SISSINGH 1977  
*Calculites ovalis* (STRADNER 1963) PRINS et SISSINGH 1977  
*Ceratolithoides aculeus* (STRADNER 1961) PRINS et SISSINGH in SISSINGH 1977  
*Chiastozygus litterarius* (GORKA 1957) MANIVIT 1971  
*Cretarhabdus crenulatus* BRAMLETTE et MARTINI 1964  
*Cretarhabdus conicus* BRAMLETTE et MARTINI 1964  
*Cribrocorona gallica* (STRADNER 1963) PERCH-NIELSEN 1973  
*Cribrosphaerella ehrenbergii* (ARKHANGELSKY 1912) DEFLANDRE 1952  
*Cylindralithus serratus* BRAMLETTE et MARTINI 1964  
*Eiffelithus eximius* (STOVER 1966) PERCH-NIELSEN 1968  
*Eiffelithus turriseiffelii* (DEFLANDRE et FERT 1954) REINHARDT 1965  
*Eiffelithus* cf. *gorkae* REINHARDT 1965  
*Gartnerago obliquum* (STRADNER 1963) NOEL 1970  
*Glaukolithus diplogrammus* (DEFLANDRE 1954) REINHARDT 1964  
*Heteromarginatus* sp.  
*Lithraphidites carniolensis* DEFLANDRE 1963  
*Lucianorhabdus cayeuxii* DEFLANDRE 1959  
*Lucianorhabdus cayeuxii* DEFLANDRE 1959 ssp. B, WAGREICH 1988  
*Manivitella pemmatoidea* (DEFLANDRE in MANIVIT 1965) THIERSTEIN 1971  
*Microrhabdulus decoratus* DEFLANDRE 1959  
*Micula decussata* VEKSHINA 1959  
*Micula praemurus* (BUKRY 1973) STRADNER et STEINMETZ 1984  
*Ottavianus giannus* RISATTI 1973  
*Placozygus fibuliformis* (REINHARDT 1964) HOFFMANN 1970  
*Prediscosphaera cretacea* (ARKHANGELSKY 1912) GARTNER 1968  
*Prediscosphaera spinosa* (BRAMLETTE et MARTINI 1964) GARTNER 1968  
*Quadrum gartneri* PRINS et PERCH-NIELSEN in MANIVIT et al. 1977.  
*Quadrum gothicum* (DEFLANDRE 1959) PRINS et PEARCH-NIELSEN in MANIVIT et al. 1977  
*Quadrum siissinghii* PEARCH-NIELSEN 1986  
*Quadrum trifidum* (STRADNER in STRADNER et PAPP 1961) PRINS et PEARCH-NIELSEN in MANIVIT et al. 1977  
*Reinhardtites anthophorus* (DEFLANDRE 1959) PERCH-NIELSEN 1968  
*Reinhardtites levis* PRINS et SISSINGH in SISSINGH 1977  
*Rhagodiscus angustus* (STRADNER 1963) REINHARDT 1971  
*Rhagodiscus splendens* (DEFLANDRE 1953) VERBEEK 1977  
*Russellia multiplus* (PERCH-NIELSEN 1973) WIND et WISE 1977  
*Rucinolithus* sp. (6-rajed)  
*Scampanella cornuta* FORCHHEIMER et STRADNER 1973  
*Tranolithus orionatus* (REINHARDT 1966) PERCH-NIELSEN 1968  
*Vekshinella stradneri* ROOD et al. 1971  
*Watznaueria barnesae* (BLACK 1959) PERCH-NIELSEN 1968  
*Zeugrhabdotus embergeri* (NOEL 1959) PERCH-NIELSEN 1984



### Palaeoenvironmental conclusions

According to the latest palaeogeographic maps, the boundary of the the mediterranean and the boreal region of the Tethys developed at the border of the subtropical and polar oceanic fronts during the Late Cretaceous (HAY et al., 1994). This boundary was situated in the territory of present-day Central Europe. The extension of the Tethys was about three times greater than that of the present Mediterranean Sea. It was warm and shallow (HAY, 1994). On its territory rich in archipelagoes developed corresponding to the changes of the sealevel. The Hungarian Senonian formations were deposited in the northern archipelagic area of the Tethys. The dry lands had abundant fern, pine and diversified, predominantly angiospermous (Normapolles) vegetation. On the surrounding elevated relief, the erosion of Carboniferous and Upper Triassic formations took place. In the sea lived abundant phytoplankton assemblages (dinoflagellates, nannoplankton).

On the basis of the massive occurrence of the rich sporomorphs remnants, the sedimentation took place in the shallow marine and neritic nearshore region.

This is also proved by the consistent occurrence of *Scolecodonta* (Annelidae), remnants of bottom dweller worms.

Besides the abundant vegetation, the tropical-subtropical climate is also indicating by the common occurrence of thermophilic *Belemnites*.

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## GENERAL CONNECTIONS BETWEEN LATEX AND NECTAR SECRETIONAL SYSTEMS OF *ASCLEPIAS SYRIACA* L.

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(Received: May 22, 1995)

### Abstract

The intracellular latex secretion and the extracellular nectar secretion are anatomically connected to each other in *Asclepias syriaca* L. The whole gynostemium is interwoven by the non-articulated laticifers, and they can also be found in the tissues of the epimorph nectary. The latex system is divided into several branches in the gland parenchyma, and the epithelial glandular tissues of the nectary. The question as to whether there is also a functional connection as a consequence of the anatomical connection (or whether it is possible that certain materials of the latex are secreted into the nectar and are finally mixed into the honey) between the two secretional systems was answered by TLC and GC-MSD analyses of the latex and the honey.

By means of TLC analyses at 254 nm and reactions with general alkaloid reagents (Dragendorff reagent, Meyer reagent and 1% Ce(SO<sub>4</sub>)<sub>2</sub> solution in 1 M H<sub>2</sub>SO<sub>4</sub>), several compounds were detected both in the latex and in the honey of *Asclepias*. Due to the lack of TLC standards, these compounds were not identified, and only the degree of compositional identity of the latex and the honey was established.

By means of GC-MSD (a gas chromatograph coupled with a mass selective detector) analyses, the following compounds which can also be found in the latex were identified in the honey of *Asclepias syriaca*: 2-propenoic acid 6-methylheptyl ester (retention time: 6.95 min), pentadecane (ret. time: 10.80 min), diethyl phthalate (ret. time: 11.43 min), hexadecane (ret. time: 12.05 min), heptadecane (ret. time: 13.20 min), 2,6,10,14-tetramethyl pentadecane (ret. time: 13.30 min), octadecane (ret. time: 14.32 min), 2-tetradecanoic acid methyl ethyl ester (ret. time: 14.45 min), phthalic acid butyl 2-methylpropyl ester (ret. time: 15.56 min), 1-eicosene (ret. time: 17.15 min), docosane (ret. time: 18.30 min) and *bis* (2-ethylhexyl) phthalate (ret. time: 20.94 min).

The functional connection between the two secretional systems consequent from their anatomical connection is strongly suggested by the partial compositional identity of the latex and the honey, and the direct connection of the latex-system with the glandular tissue of the nectary.

**Key words:** secretion, nectary, honey, *Asclepias*.

### Introduction

In the past few decades, a number of data have been obtained regarding the nectar production of melliferous plants, the quantity of honey produced and its composition. The chemical composition of the nectar produced, and finally the honey, is an important medical question. In the case of plants that produce latex, it can be influenced consider-



ably by the connection of intracellular and extracellular secretion. This paper examines the anatomical and functional connection of the intracellular latex secretion and the extracellular nectar secretion of *Asclepias syriaca*, which is an important plant in bee-farming.

It is a well-known fact that the common milkweed is a plant that contains latex. The latex can be found in long, expanded, non-articulated laticifers as the product of intracellular secretion (METCALFE, 1985). In fact, the latex can be regarded as the cell fluid of laticifers (FREY-WYSSLING, 1933; ESAU, 1953), which may contain several biologically active compounds such as organic acids, terpenoids (ADAMS, 1987), alkaloids (SOKOLOV, 1952), saturated and aromatic hydrocarbons (CAMPBELL, 1983; SIMIONESCU, 1987), steroids and alcohols (BISBOAR, 1983), in the form of either solution or colloidal suspension. The chemical compounds of the *Asclepias* genus are also mentioned by HEGNAUER (1966).

In contradiction with the statements of SPRENGEL (1793), STADLER (1886), KNUTH (1909), FITTING (1930) and RENDLE (1953) regarding the nectar-secretional system of the common milkweed, GALLIL and ZERONI (1965) and KEVAN et al. (1989) provided information on the anatomy and tissue structure of the nectar-secretional system of *Asclepias* species, and the epithelial position of the glandular tissue, which is so important here.

The composition of the nectar produced by extracellular secretion, and finally the composition of the honey are extremely diversified. Besides the most important compounds, which are different types of carbohydrates (SOUTHWICK, 1981, 1983a,b), the nectar may contain vitamins (WEBER, 1942), other organic acids (MAURIZIO, 1960), antimicrobial compounds (KEVAN et al., 1989), etc. The toxic compounds of the nectar were submitted to detailed examinations by SCHULTZ-LANGER (1966, 1967) among others. In the world of plants there also occur honeys which have a harmful, toxic effect on the human organism. The honey of some tropical *Euphorbia* species has a strong, irritant effect (JURITZ, 1925). Compounds with the same physiological effect were also detected in the latex of these plants (UPADHYAY and HECKER, 1975; SOSATH, 1988).

The common origin of certain compounds of nectar and latex, and the connection between extracellular and intracellular secretion in the *Euphorbia* genus, have already been emphasized in previous publications (TÓTH-SOMA and GULYÁS, 1991; TÓTH-SOMA et al., 1993). In the present paper, the following questions are examined: is the latex system of *Asclepias syriaca* anatomically connected to its nectar-secreting system, and if it is, is there also a functional connection between them due to their anatomical connection? May certain compounds of the latex be secreted into the nectar, and finally into the honey?

## Materials and methods

### *Anatomical examinations on the nectary.*

The tissue structure of the nectaries in the stigmatic chambers of the gynostemium of *Asclepias syriaca* was examined; this is an excellent melliferous plant found all over Europe. To examine the gynostemium derived from its petals, we used the celloidin embedding method described by KISSER (1920) and ROMEIS (1932), as modified by GULYÁS (1968). We made 20 to 30 µm thick cross-sections with sledge-microtome. For better examination of the gland, the cross-sections were stained with Erlich's haematoxylin and conserved in Canada balsam. The slides were examined under an NU-2 light microscope.

### *Chemical detections*

The comprehensive analyses of milkweed latex and honey were performed by analytical techniques. By means of thin-layer chromatography, alkaloids and other compounds were detected which gave a positive reaction with alkaloid reagents. Other organic compounds were detected by the GC-MSD method.

From the latex and honey, a chloroform extract was made according to the method applied by SZÁSZ (1979) and TÓTH-SOMA et al. (1993). These extracts were examined on Kieselgel 60F 254 (MERCK) thin-layer plates, by developing the chromatograms in an 85:15 (v/v) mixture of benzene and methanol. Preliminary examination of the plates was performed under 254 nm light. They were then developed with three general alkaloid reagents: a 1% solution of  $\text{Ce}(\text{SO}_4)_2$  in 1 M  $\text{H}_2\text{SO}_4$ , the Meyer reagent and the Dragendorff reagent (HAIS and MACEK, 1961; MUNIER and MACHEBOEUF, 1949). The extracts were further examined with a Hewlett Packard GC-MSD system (an HP 5890A GC coupled to an HP 5970 MSD, 70 eV EI), using a 12 m × 0.2 mm × 0.5 µm HP-1 capillary column (OV-1 compatible), and He as carrier gas.

## Results

During the anatomical examinations of the nectaries, their structure (already predicted in the literature) was observed. In the light microscopical pictures, the differentially stained gland-parenchyma and the epithelial glandular tissue could be clearly observed (Fig. 1, panel A). The whole gynostemium and both layers of the parenchyma of the epimorph nectary situated in the stigmatic chamber are interwoven by the inarticulate system of laticifers which is characteristic of the plant (Fig. 1, panels A, B, C). The nectar stored in the stigmatic chamber is responsible for the germination of the pollinium that comes from outside (Fig. 1, panel D). The laticifers closer to the epithelial glandular tissue are parallel with the secretional layer of the nectary (Fig. 1, panel E), and their final ramifications end on the border between the subepithelial parenchyma and the epithelial tissue, directly touching the secretional tissue (Fig. 1, panel F). It is clear, therefore, that the intracellular latex secretion and the extracellular nectar secretion are anatomically connected to each other.

The functional connection resulting from the anatomical connection was verified by TLC examinations. These examinations revealed that several compounds that are present in the latex are also present in the honey (Fig. 2). On the chromatograms examined under 254 nm light, four compounds were found which were present in the extracts of both honey and latex (Fig. 2, spots a-d, plate I). On the plate developed with Meyer reagent, there were five compounds (Fig. 2, spots a-e, plate II), the plate

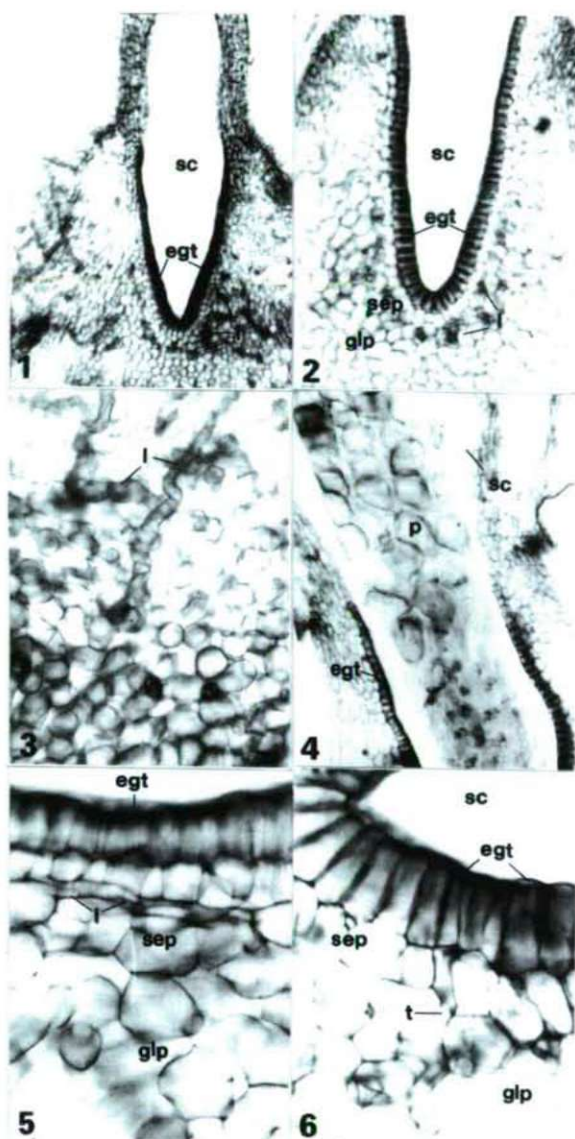


Fig. 1. Cross-section of the floral nectaries of *Asclepias syriaca* L. Enlargement: panel A 80 $\times$ , B 125 $\times$ , C 265 $\times$ , D 125 $\times$ , E 500 $\times$ , F 530 $\times$ , epithelial glandular tissue (egt), subepithelial parenchyma (sep), gland-parenchyma (glp), laticifers (l), stigmatic chamber (sc), pollinary (p). See explanation in text.



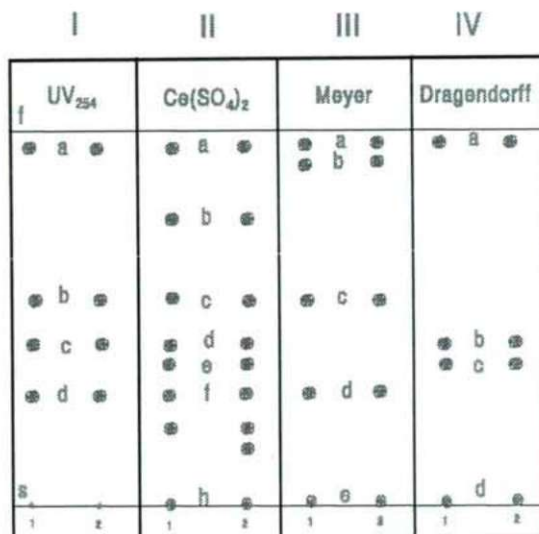


Fig. 2. TLC of latex (1) and honey (2) extracts of *Asclepias syriaca* L. The distances of the spots from the front from a to h. 1-2: numbers of samples, f: front, s: start.

developed with  $\text{Ce}(\text{SO}_4)_2$  revealed eight compounds (Fig. 2, spots a-h, plate III), and on the plate developed with Dragendorff reagent, there were four compounds (Fig. 2, spots a-d, plate IV) that could be found in both extracts. Due to the lack of TLC standards, we did not investigate these compounds qualitatively, but the compositional identity of the latex and the honey was thus clearly demonstrated.

In the GC-MSD examinations, several identical compounds could be observed (Fig. 3). The mass spectra (with the help of the NBS mass spectrum library) permitted identification of the following compounds in the honey extract (Fig. 3, B): 2-propenoic acid 6-methylheptyl ester (ret. time: 6.95 min), pentadecane (ret. time: 10.80 min), diethyl phthalate (ret. time: 11.43 min), hexadecane (ret. time: 12.05 min), heptadecane (ret. time: 13.20 min), 2,6,10,14-tetramethyl pentadecane (ret. time: 13.30 min), octadecane (ret. time: 14.32 min), 2-tetradecanoic acid methyl-ethyl ester (ret. time: 14.45 min), phthalic acid butyl 2-methylpropyl ester (ret. time: 15.56 min), 1-eicosene (ret. time: 17.15 min), docosane (ret. time: 18.30 min) and *bis* (2-ethylhexyl) phthalate (ret. time: 20.94 min). The peaks of these compounds can also be seen on the gas chromatogram of the latex extract (Fig. 3, A). As these peaks appeared at the same retention times with the same mass spectra for both extracts, they are considered to relate to identical compounds.

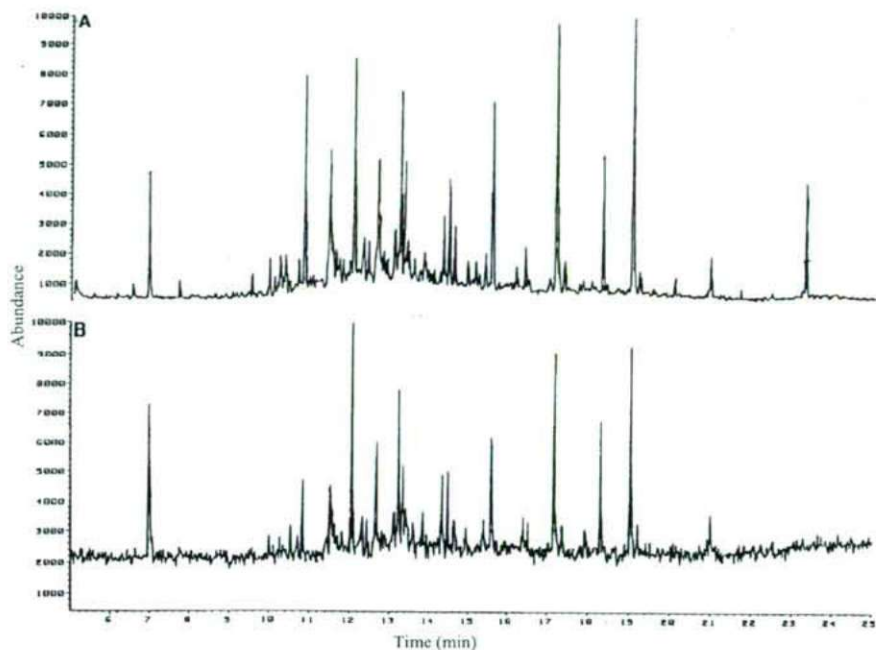


Fig. 3. Gas chromatograms of latex (A) and honey (B) extracts of *Asclepias syriaca* L.

### Discussion

Our anatomical examinations of the gynostemium and the nectary verify that the intracellular latex-secretional and the extracellular nectar-secretional systems of *Asclepias syriaca* L. are anatomically connected to one another. The parenchyma layer of the nectary is richly interwoven by inarticulated laticifers which are also present in the gynostemium and are characteristic of the whole plant. After several ramifications, the laticifers end on the border between the epithelial glandular tissue and the gland-parenchyma of the nectary. Therefore, a functional connection between the two secretional system is anatomically possible. This assumption was strongly indicated by the TLC and GC-MSD analyses of the honey and the latex. Our results harmonize with the anatomical observations made so far on the nectary (GALID and ZERONI, 1965; KEVAN et al. 1989) supplemented by the position of the laticifers in the gynostemium compared to their glandular tissue. Our results provide new data concerning the observation of the connection between the two secretional systems mentioned above.

Our results suggest that the *Asclepias* honey sold commercially should be examined medically and toxicologically.

### Acknowledgements

We are grateful to Professor C. P. BHAUMIK for his critical reading of the manuscript and his help with the publication of this paper.

This work was supported by National Scientific Research Found (Grant number F2 F0060012).

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## CRANIAL OSTEOLOGY OF THE SAND GOBY *NEOGOBIOUS FLUVIATILIS* (PALLAS, 1881) FROM THE RIVER SAVA (SERBIA, YUGOSLAVIA)

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(Received: May 8, 1996)

### Abstract

The intrapopulation variability of the sand goby *Neogobius fluviatilis* (PALLAS, 1811) from the River Sava mostly corresponds to the species intraspecific variability reported previously. Certain characters continuously increase through four examined size classes, while others increase most intensively in the largest size class, whereas the size of the suspensorial opening varies significantly, but irregularly. Allometry was not detected within the examined size range of sand gobies. There were no significant morphological differences between successive size classes. The influence of size on shape was negligible. The most variable trait, representing the metapterygoid process, varied stochastically, thus implying that it is of low interest for phylogenetic considerations. Other variable traits mostly describe elements of both upper and lower jaws and gill cover. The changes detected in skull elements (sphenotic and pterotic) are not strongly expressed, due to their small participation in the overall variability of the sample. It seems that gobies over 11 cm in total length are more diverse morphologically than those below that size.

*Key words:* sand goby, cranial osteology, morphology, intrapopulation variability.

### Introduction

Ponto-Caspian gobiids, of the genera *Neogobius* ILJIN 1927, *Proterorhinus* SMITH 1899, *Mesogobius* BLEEKER 1874, *Knipowitchia* ILJIN 1927, *Benthophilodes* BELING and ILJIN 1927 and *Benthophilus* EICHWALD 1831, have been investigated mostly by Russian and Ukrainian ichthyologists. Papers on the genus *Neogobius* by ILJIN (1927, 1949), BERG (1949), PINCHUK (1963, 1976, 1977, 1991) and SVETOVIDOV (1964) dealt mostly with traditional morphological characters of the gobiids, as follows: the seismosensory system of the modified lateral system on the head, the numbers of spines and rays in the fins, the form and structure of the pelvic disc, the form of the dorsal fins, the scale type on the nape and body, the number of scale rows on the body, etc. These reports elucidated the position of the genus *Neogobius*, and enabled most authors to establish the subgeneric division of this genus to the subgenera *Apollonia* ILJIN, 1927; *Ponticola* ILJIN, 1927; *Babka* ILJIN, 1927; and *Neogobius* BERG, 1949. This division was mostly accepted, although some other subgenera were also introduced, e.g.

*Eichwaldiella* WHITLEY, 1930 and *Chazar* ILJIN in BERG, 1949 (PINCHUK, 1991), comprising the Caspian Sea species the status of which is as yet insufficiently known.

Osteological investigations on the genus *Neogobius* have mostly been limited to reports on the vertebra number. The cranial osteology was not studied until the work of VASILEVA (1988), who investigated most species of this genus.

According to ILJIN (1927, 1949), BERG (1949), PINCHUK (1963, 1976, 1977, 1991) and SVETOVIDOV (1964), the subgenus *Neogobius* is monotypic, with only one species, the sand goby, *N. fluviatilis* PALLAS 1811. Recent papers (VASILEVA, 1988, 1989) on cranial osteology rejected this subgenus, and classified the sand goby together with the round goby *N. melanostomus* (PALLAS, 1811) in the subgenus *Apollonia*.

The intraspecific osteological variability of the sand goby was reported by VASILEVA (1988). That paper dealt with different qualitative character states within particular populations for several traits, as well as with the interpopulation variability for the frequencies of these character states. Several traits, different for particular populations, were also reported as variable regarding the size of the investigated specimens, and the sexual dimorphism was also quoted for some characters. However, there was no detailed information on the significance of the reported differences. Various qualitative qualifications (e.g. "undoubtedly different" or "differences not great") were given instead. Further, there was no difference in skull structure between the Azov Sea (*N. fluviatilis fluviatilis*) and the Caspian Sea (*N. f. pallasi*) sand gobies. The final conclusion from these investigations was that the "intraspecific variability of the skull of the sand goby is not great, which implies that craniological characteristics of the species are sufficiently compact" (VASILEVA, 1988).

The morphometric investigation of the cranial osteology of a particular species of the genus *Neogobius* has the aim of contributing to their classification. This aim is of further interest as regards the western distribution area of the River Sava sand goby population, which is advantageous for a comparison with populations from the distribution centre of this species. Therefore, an investigation of the intraspecific variability of the sand goby is useful for making decision concerning the use of particular characters in the phylogenetic analysis of the genus.

### Material and methods

Specimens for osteological analysis were caught by angling (hook size 12-16), mostly onshore, at a depth of 0.5-1.5 m, on the River Sava left bank, app. 1.5-2.5 km upstream from its mouth into the River Danube, in the Belgrade area, during 1994. A total of 16 specimens were analyzed. Their sex was not determined. Their total length varied between 95.8 mm and 135.4 mm.

The preparation of skeletons was as follows: skinning, flesh removal by *Dermestes lardarius* (Dermestidae, Coleoptera), a short bleaching (3% H<sub>2</sub>O<sub>2</sub>) and hot water immersion (for app. 30 minutes, depending on the size of a skeleton) for decomposition of splanchnocranium, shoulder girdle and operculum. 50 osteological characters were measured description and abbreviation of which is summarized in Table 1. They were processed as follows:

By descriptive statistics on indices for the whole data set, in order to allow comparison with the results presented in VASILEVA (1988). Characters on the skull skeleton were indexed vs. the skull base length, as



well as characters of other head skeleton units (suspensorium, jaw apparatus, gill cover and pectoral girdle). Characters of particular bone parts were indexed on the respective bone length.

By unifactorial ANOVA (SOKAL and ROHLF, 1981), on indices for 4 size classes. The first one (1) comprised specimens smaller than 10 cm in total length; the second one (2) contained specimens with a total length between 10 cm and 10.9 cm; the total length range of the third size class (3) specimens was 11-11.9 cm; while specimens of the fourth size class (4) had a total length over 12 cm.

By multivariate methods on the logarithmic-transformed raw data set (SNEATH and SOKAL, 1973), i.e. by sheared principal component analyses on variance-covariance matrix (BOOKSTEIN *et al.*, 1985), and by UPGMA clustering of Manhattan distances between sheared principal component score centroids of size classes. Scores from the covariance matrix analysis served for the calculation of ontogenetic trajectories, according to HUMPHRIES *et al.* (1981), and their multiple comparison between successive size classes by TUKEY'S *q*-test (ZAR, 1984), whereas loadings from an ordination of the correlation matrix were used for an inspection of allometry, according to JOLICEUR (1963).

## Results

The head skeleton includes a moderately high skull, with a straight posterior part of the roof, and a curved descent on the anterior part, from the rear edge of the orbit to the tip of the vomer (Fig. 1). The greatest width of the skull is at the pterotic level, and the smallest at the level of the exoethmoid (Fig. 2). The dental is relatively short and high, especially at its rear end, with no prominent teeth at the rear end of the rather long dental row. The rear, lower part of the articular is low (less than 1/5 of the bone height) and short (less than 2/3 of the bone length). The praemaxillar is relatively short and high. The anterior articulating surface of the palatine is well developed and comprises more than half of the whole bone length. The height of the hyomandibular is approximately equal to its length. The rear, stout part of the praeoperculum is relatively wide, and angle- or almost boomerang-shaped, with a narrow, long "handle". The ventral part of the cleithrum is relatively short and stout (Fig. 3).

A comparison of our results (Table 1) with the data of VASILEVA (1988) revealed that the sand goby from the River Sava is similar to the sand goby from the Azov Sea in Spt, Wpraeop, 12praemax, lhyom, Whyoman and Wcleit; it has bigger values for Hmax, Lforus, Lorbit, lsubop, wpraeop, lpraeop, Hdent, latric and Hartic; and smaller values for all other characters. In many characters (e.g. Spt, Lforus, Sarcident, Lprocsph, Lorbit, Wopec, Wab, Wsubop, lsubop, Wpraeop, wpraeop, lpraeop, Hdent, ldent, latric, hartic, Hpraemax and hcpraemax), the sand goby from the River Sava is more similar to those from the different Caspian Sea tributary populations than to the Azov Sea sand goby.

The variability between the four established size classes (Table 2) was not marked for most of the osteological traits of the cranium. Nevertheless, several characters revealed a continuous increase, e.g. Ssp, Spt, Lprocsph and lsubop. This increase was regular through all examined size classes. A few characters (e.g. Hartic and hartic) varied, i.e. they increased significantly between the largest size classes only, while the previous increase was insignificant. Only one of the characters, i.e. Lforus, decreased at first, and thereafter increased significantly.

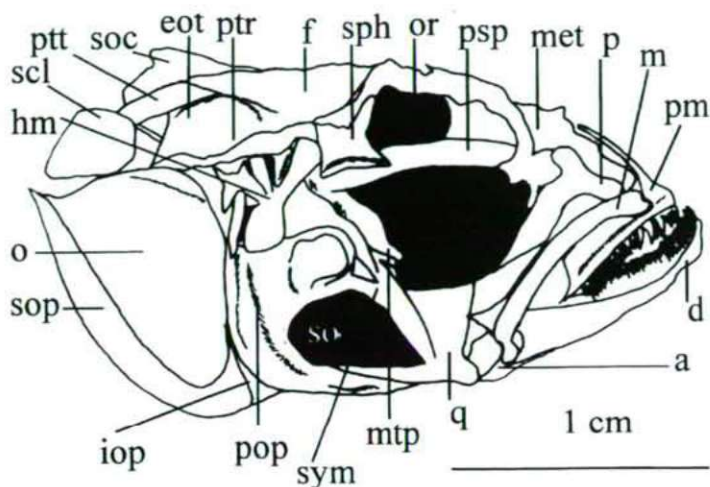


Fig. 1. The cranium of the sand goby, including the operculum and pectoral girdle - a gross view (a: articular; d: dental; eot: epiotic; f: frontal; hm: hyomandibular; iop: interoperculum; m: maxillar; mtp: metapterygoid; o: operculum *s. stricto*; or: orbit; p: palate; pm: praemaxillar; pop: praeoperculum; psp: parasphenoid; ptr: pterotic; ptt: posttemporal; q: quadrate; so: an opening between suspensorium (i.e. quadrate, metapterygoid and symplectic) and praeoperculum; scl: supracleithrum; soc: supraoccipital; sop: suboperculum; sph: sphenotic).

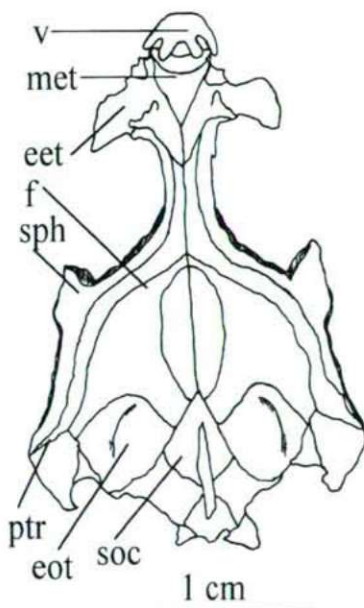


Fig. 2. Dorsal side of the cranium of *Neogobius fluviatilis* (eet: exoethmoid; v: vomer; other symbols as in Fig. 1).

Table 1. Descriptive statistics results (min = minimum; max = maximum; M = mean; s = standard error of mean; CV = variation coefficient) for the osteological characters of *Neogobius fluviatilis* (n = 16).

Character	Description	min	max	M	s	CV
Lcran	skull base length	16.4	22.2	18.9	0.4	9.0
Hmax	maximal height of skull at level of supraoccipital crest	26.6	30.8	28.4	0.3	4.2
Hm	skull height at mesethmoid level	10.7	15.5	13.1	0.3	9.1
Hfr	skull height at frontal level	22.2	25.7	23.8	0.2	3.9
Sprf	skull width at exoethmoid level	28.3	39.1	33.5	0.8	9.0
Ssp	skull width at sphenotic level	47.3	53.6	49.2	0.4	3.5
Spt	maximal skull width at pterotic level	63.7	70.3	66.3	0.5	2.9
Lforsus	greater diagonal width of suspensorial opening	23.7	26.2	25.0	0.2	3.1
Lpraeorb	praeorbital length	13.6	18.0	16.0	0.3	7.3
Sarcdent	width of intact dentary arch at articulare-quadratum joint level	26.8	41.9	33.2	0.9	10.3
Lprocsph	sphenotic extension length	10.0	14.0	11.3	0.3	9.8
Lsphpt	sphenotic-pterotic length	42.3	47.8	44.8	0.3	3.0
Lorbit	diagonal orbit length	31.5	37.5	33.8	0.4	4.5
Loperc	rear opercular edge length	44.6	61.7	49.4	1.1	9.3
Woperc	maximal opercular width	38.0	63.2	52.8	3.5	13.2
Lsubop	subopercular width	55.7	64.0	59.4	0.6	4.1
Lsubop	length of lower anterior subopercular extension	37.1	49.6	42.0	0.8	7.2
Wsubop	subopercular width	19.0	34.4	25.0	1.0	16.0
Lpraeop	length of praeopercular	55.0	66.5	59.0	0.8	5.1
lpraeop	length of lower praeopercular extension that joins quadrate	38.5	52.5	45.6	0.9	7.7
Wpraeop	maximal praeopercular width	36.8	48.2	44.0	0.8	7.4
wpraeop	praeopercular width without anterior middle extension	30.9	38.5	35.2	0.5	5.9
Ldent	length of dentary	34.5	49.8	40.0	0.8	8.0
ldent	length of teeth row	58.6	74.4	65.9	1.1	7.0
Hdent	rear maximal height of dentary	37.7	48.3	40.6	0.7	7.1
hdent	height of dentary at level of rear end of teeth row	17.8	25.7	20.9	0.4	8.6
Lartic	maximal length of articular	33.2	40.2	36.4	0.5	5.6
lartic	length of lower palate of articular	52.7	64.9	59.7	0.8	5.7
Hartic	maximal height of articular	38.1	56.3	43.4	1.1	9.7
hartic	height of lower palate of articular	15.5	21.4	18.6	0.4	8.8
Lpraemax	length of praemaxilla	28.3	35.3	30.1	0.5	6.1
l1praemax	length of depressed middle part of praemaxilla ridge	11.1	32.7	16.7	1.3	29.9
l2praemax	length of the elevated rear part of praemaxilla ridge	40.4	51.7	45.4	0.9	8.3
Hpraemax	maximal height of praemaxilla	58.6	68.1	62.8	0.7	4.5
hpraemax	height of praemaxilla articulation surface for joint with skull	27.5	36.7	33.2	0.7	8.0
hpraemax	height of elevated rear part of praemaxilla ridge	18.3	24.5	21.8	0.4	8.1
Lpalat	length of palatine	23.8	32.6	26.4	0.5	7.9
lpalat	length of palatine front articulation surfaces	58.6	75.6	67.6	1.0	5.9
Lab	length of ventral part of last gill arch	56.0	77.0	63.1	1.3	8.3
Wab	width of ventral part of last gill arch	14.0	21.1	17.8	0.4	9.7
Lcleit	cleithrum length	79.2	90.7	84.5	0.8	3.9
lcleit	length of ventral part of cleithrum	47.0	59.7	51.6	0.7	5.6
Wcleit	cleithrum width	15.1	21.0	17.3	0.4	9.0
Lhyomand	length of hyomandibular, praeopercular extension included	30.0	40.0	32.8	0.6	6.9
lhyomand	length of hyomandibular, without praeopercular extension	104.8	132.3	115.7	1.7	5.8
Whyomand	maximal length of hyomandibular	79.7	109.2	102.1	1.6	6.4
l1/l2ptemp	length of ventral extension of posttemporal	54.0	83.1	68.1	2.1	12.6
Lmaxil	length of maxilla	35.1	41.9	37.2	0.5	5.1
lmaxil	length of front articulation surfaces of maxillary bone	27.0	43.3	35.5	1.3	14.9
Lmpt	length of metapterygoid	20.2	32.1	26.3	0.7	10.7
lmpt	length of lower metapterygoid extension	0.0	14.7	5.5	0.9	63.1



Table 2. Unifactorial ANOVA results for the comparison of particular characters (for indices see Table 1) through size classes (<10 cm; 10-11 cm; 11-12 cm; >12 cm) of *Neogobius fluviatilis* (M = mean; s = standard error of mean; F = F-value; p = probability: \*\*\* = 0.01; \*\* = 0.02; \* = 0.05; df = degree of freedom).

Character	<10 cm M ± s n = 4	10-11 cm M ± s n = 4	11-12 cm M ± s n = 4	>12 cm M ± s n = 4	F df = 3	p <
Ltot (mm)	98.60 ± 0.99	105.5 ± 0.51	115.6 ± 1.79	126.8 ± 3.06	51.90	***
Lcran	17.10 ± 0.44	17.98 ± 0.19	19.33 ± 0.60	21.03 ± 0.59	12.30	
Hmax	28.02 ± 0.67	29.36 ± 0.45	28.25 ± 0.90	28.08 ± 0.24	1.06	
Hm	12.57 ± 0.62	13.08 ± 0.43	12.63 ± 0.56	13.94 ± 0.76	1.10	
Hfr	23.54 ± 0.15	23.79 ± 0.48	24.02 ± 0.70	23.90 ± 0.61	0.10	
Sprf	32.04 ± 0.51	31.17 ± 1.76	35.73 ± 1.68	35.13 ± 0.98	2.78	
Ssp	48.40 ± 0.73	47.99 ± 0.37	49.42 ± 0.65	51.17 ± 0.95	4.02	*
Spt	64.45 ± 0.36	65.79 ± 0.77	66.48 ± 0.55	68.54 ± 0.99	5.84	**
Lforsus	25.16 ± 0.37	25.04 ± 0.14	24.08 ± 0.31	25.68 ± 0.31	5.10	**
Lpraeorb	15.74 ± 0.63	15.58 ± 0.82	16.15 ± 0.51	16.49 ± 0.54	0.41	
Sarcdent	30.69 ± 1.33	32.50 ± 1.52	32.53 ± 1.16	36.96 ± 1.73	3.39	
Lprocsph	10.38 ± 0.12	11.13 ± 0.27	11.30 ± 0.53	12.56 ± 0.68	3.88	*
Lsphpter	43.98 ± 0.62	45.48 ± 0.86	45.32 ± 0.56	44.32 ± 0.70	1.12	
Lorbit	34.99 ± 0.62	34.48 ± 1.07	32.71 ± 0.32	33.10 ± 0.56	2.42	
Loperc	51.40 ± 2.75	46.58 ± 0.69	47.80 ± 1.07	51.93 ± 3.43	1.33	
Woperc	50.92 ± 5.16	41.10 ± 12.75	52.71 ± 2.50	52.33 ± 4.00	0.25	
Lsubop	61.41 ± 1.49	57.98 ± 0.85	59.93 ± 1.01	58.14 ± 1.08	2.06	
Lsubop	39.23 ± 0.89	40.75 ± 0.60	43.21 ± 1.30	44.90 ± 1.71	4.41	*
Wsubop	22.98 ± 1.71	24.16 ± 2.23	25.87 ± 1.96	27.08 ± 2.54	0.72	
Lpraeop	58.62 ± 0.58	58.15 ± 1.28	57.84 ± 1.00	61.32 ± 2.56	1.07	
Lpraeop	47.46 ± 2.03	48.28 ± 1.28	44.30 ± 0.56	42.45 ± 1.76	3.24	
Wpraeop	42.83 ± 1.37	45.19 ± 1.39	44.60 ± 1.67	43.57 ± 2.54	0.34	
wpraeop	33.10 ± 0.91	34.70 ± 0.95	36.25 ± 0.95	36.61 ± 0.69	3.32	
Ldent	38.27 ± 1.29	39.07 ± 0.62	39.93 ± 1.01	42.61 ± 2.66	1.41	
ldent	64.82 ± 3.00	65.62 ± 2.10	64.88 ± 1.56	68.14 ± 3.13	0.38	
Hdent	41.87 ± 2.24	40.99 ± 1.65	38.64 ± 0.37	40.73 ± 1.12	0.82	
hdent	21.93 ± 1.52	19.96 ± 0.74	2.77 ± 0.64	21.12 ± 0.59	0.73	
Lartic	36.07 ± 1.02	37.27 ± 0.71	36.21 ± 1.47	36.00 ± 1.18	0.28	
lartic	61.69 ± 1.53	58.21 ± 0.81	59.23 ± 3.08	59.85 ± 0.77	0.65	
Hartic	42.59 ± 0.79	40.29 ± 1.27	41.98 ± 1.06	48.76 ± 2.56	5.52	**
hartic	17.37 ± 0.61	17.94 ± 0.94	18.65 ± 0.62	20.49 ± 0.32	4.22	*
Lpraemax	29.62 ± 0.83	29.6 ± 0.68	29.53 ± 0.49	31.68 ± 1.42	1.29	
l1praemax	16.45 ± 1.32	15.09 ± 1.31	21.57 ± 3.96	13.69 ± 1.55	2.19	
l2praemax	44.27 ± 1.18	42.29 ± 1.19	47.29 ± 1.77	47.87 ± 2.50	2.25	
Hpraemax	63.68 ± 0.94	62.94 ± 1.48	60.11 ± 0.66	64.45 ± 1.86	2.06	
hpraemax	33.57 ± 1.78	30.99 ± 1.53	34.15 ± 1.19	34.13 ± 0.42	1.28	
hpraemax	21.15 ± 1.12	22.17 ± 0.96	21.10 ± 1.19	21.73 ± 0.52	0.25	
Lpalat	26.00 ± 0.58	26.70 ± 1.16	25.59 ± 0.61	27.43 ± 1.74	0.52	
lpalat	67.37 ± 0.53	66.46 ± 2.25	69.79 ± 2.25	66.77 ± 2.91	0.48	
Lab	60.25 ± 2.46	62.19 ± 2.24	64.81 ± 1.92	65.01 ± 4.09	0.66	
Wab	17.20 ± 0.64	17.29 ± 1.41	18.34 ± 0.62	18.56 ± 0.88	0.56	
Lcleit	82.09 ± 1.32	82.77 ± 0.64	86.33 ± 1.86	86.94 ± 1.65	2.88	
lcleit	51.17 ± 1.03	51.78 ± 1.71	52.05 ± 2.62	51.41 ± 0.35	0.06	
Wcleit	17.05 ± 0.41	17.14 ± 0.35	16.40 ± 0.65	18.67 ± 1.26	1.60	
Lhyomand	31.85 ± 0.53	32.27 ± 0.58	32.23 ± 0.50	34.79 ± 2.06	1.43	
lhyomand	117.7 ± 3.40	114.3 ± 1.83	114.2 ± 3.48	116.8 ± 5.52	0.23	
Whyomand	103.2 ± 1.48	101.5 ± 1.68	104.6 ± 1.65	99.19 ± 6.63	0.42	
l1/l2ptemp	68.67 ± 5.23	68.84 ± 4.13	67.38 ± 4.01	67.50 ± 6.10	0.02	
Lmaxil	35.94 ± 0.38	36.72 ± 0.53	37.01 ± 0.77	39.09 ± 1.40	2.43	
lmaxil	34.36 ± 2.27	37.00 ± 3.48	37.03 ± 3.35	33.62 ± 2.35	0.37	
Lmptpr	25.11 ± 1.64	24.85 ± 1.54	27.06 ± 0.61	28.15 ± 1.60	1.26	
lmptpr	6.31 ± 2.93	2.93 ± 1.15	6.14 ± 1.30	6.78 ± 1.26	0.94	

The results of the multivariate analysis demonstrated the negligible influence of the general size component. Neither allometry during the growth ( $\chi^2 = 0.300$ ;  $df = 51$ ), nor a difference in the calculated ontogenetic trajectories between size classes was detected (Table 3). The highest loading on PC1 ( $\lambda_1 = 4.480$ ; 93.89%), i.e. a size component, was that of *Imptcr*, whereas *Woperc* had the highest loadings on PC2 ( $\lambda_2 = 0.168$ ; 3.51%) and PC3 ( $\lambda_3 = 0.077$ ; 1.61%), i.e. shape components, both before and after the shearing. Some other characters, e.g. *Wsubop*, *l2praemax*, *ldent* and *hdent*, loaded PC2 highly, whereas *Sarcent*, *hartic*, *lmaxill* and *Lmptcr* loaded PC3 (Table 4).

Table 3. Ontogenetic trajectories and their standard error (diagonal) for four size classes of *Neogobius fluviatilis*, and their successive testing by TUKEY'S q test ( $k = 4$ ;  $n = 16$ ; below diagonal).

Size class	<10 cm	10-11 cm	11-12 cm	>12 cm
<10 cm	11.55 $\pm$ 6.80			
10-11 cm	3.681	-0.47 $\pm$ 0.22		
11-12 cm		2.623	14.16 $\pm$ 6.26	
>12 cm			1.053	21.66 $\pm$ 6.49

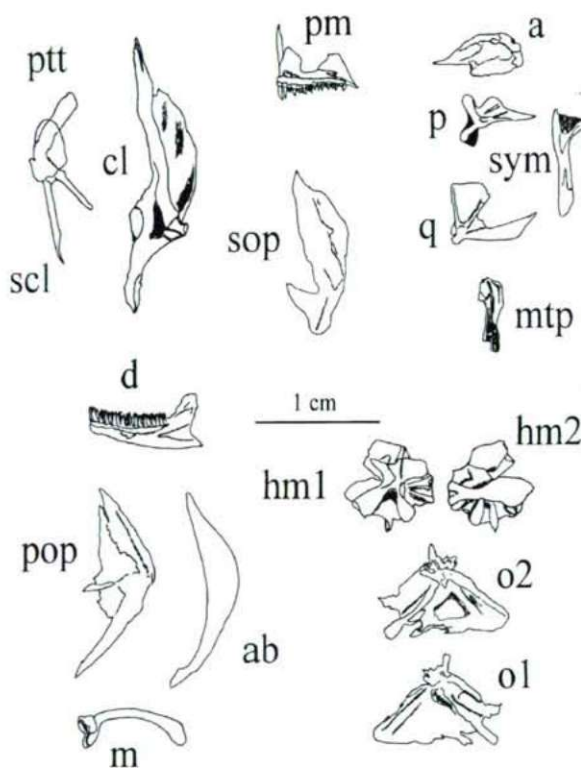


Fig. 3. Bones of the splanchnocranium, operculum *s. lato* and pectoral girdle of *Neogobius fluviatilis* (ab: fifth branchial arch; hm1: inner side, and hm2: outer side of hyomandibular; o1: outer side, and o2: inner side of operculum *s. stricto*; sym: symplectic; other symbols as in Fig. 1).

Table 4. Principal component loadings of particular osteological characters (for legends see Table 1) of *Neogobius fluviatilis* before (PC1-3) and after (H2-3) shear.

Character	PC1	PC2	PC3	H2	H3
Lcran	0.003	0.084	-0.059	0.084	-0.059
Hmax	0.002	0.087	-0.065	0.087	-0.065
Hm	0.008	0.109	-0.079	0.109	-0.079
Hfr	0.006	0.086	-0.059	0.086	-0.059
Sprf	0.006	0.145	-0.027	0.145	-0.027
Ssp	0.005	0.110	-0.064	0.110	-0.064
Spt	0.003	0.112	-0.068	0.112	-0.068
Lforsus	0.004	0.086	-0.063	0.086	-0.063
Lpraeorb	-0.001	0.145	-0.031	0.145	-0.031
Sarcdent	-0.001	0.145	-0.131	0.145	-0.131
Lprocsph	0.003	0.157	-0.084	0.157	-0.084
Lsphpter	0.000	0.092	-0.059	0.092	-0.059
Lorbit	0.005	0.044	-0.074	0.044	-0.074
Loperc	0.005	0.100	-0.059	0.100	-0.059
Woperc	-0.005	0.487	0.847	0.487	0.847
Lsubop	0.002	0.083	-0.041	0.083	-0.041
Lsubop	0.004	0.131	-0.077	0.131	-0.077
Wsubop	-0.004	0.199	-0.040	0.199	-0.040
Lpraeop	0.002	0.118	-0.060	0.118	-0.060
lpraeop	-0.002	0.056	-0.030	0.056	-0.030
Wpraeop	-0.003	0.107	-0.073	0.107	-0.073
wpraeop	0.004	0.158	-0.054	0.158	-0.054
Ldent	0.004	0.033	-0.097	0.033	-0.097
ldent	0.006	0.171	-0.081	0.171	-0.081
Hdent	0.004	0.121	-0.096	0.121	-0.096
hdent	0.005	0.167	-0.039	0.167	-0.039
Lartic	0.000	0.088	-0.073	0.088	-0.073
lartic	0.001	0.086	-0.048	0.086	-0.048
Hartic	0.005	0.146	-0.084	0.146	-0.084
hartic	0.011	0.129	-0.119	0.129	-0.119
Lpraemax	0.002	0.129	-0.068	0.129	-0.068
l1praemax	0.016	0.103	0.060	0.103	0.060
l2praemax	0.008	0.172	-0.051	0.172	-0.051
Hpraemax	0.003	0.140	-0.053	0.140	-0.053
hpraemax	0.008	0.139	-0.092	0.139	-0.092
hpraemax	0.008	0.102	-0.078	0.102	-0.078
Lpalat	-0.003	0.121	-0.076	0.121	-0.076
lpalat	0.003	0.115	-0.056	0.115	-0.056
Lab	0.004	0.134	-0.082	0.134	-0.082
Wab	0.018	0.165	-0.059	0.165	-0.059
Lcleit	0.006	0.109	-0.063	0.109	-0.063
lcleit	0.002	0.117	-0.070	0.117	-0.070
Wcleit	0.003	0.159	-0.087	0.159	-0.087
Lhyomand	0.002	0.125	-0.079	0.125	-0.079
lhyomand	0.005	0.122	-0.088	0.122	-0.088
Whyomand	0.003	0.132	-0.086	0.132	-0.086
l1ptemp	0.002	0.045	-0.090	0.045	-0.090
l2ptemp	0.002	0.079	-0.031	0.079	-0.031
Lmaxil	0.003	0.123	-0.075	0.123	-0.075
lmaxil	-0.001	0.093	-0.138	0.093	-0.138
Lmpter	0.002	0.137	-0.130	0.137	-0.130
lmpter	0.999	-0.020	0.015	-0.020	0.015



The sheared component scores (Fig. 4) and a phenogram of examined size class specimen centroids (Fig. 5), based on the UPGMA clustered Manhattan distances between them (Table 5), revealed the directed morphology formation. The most similar successive size classes were the smallest ones, while the most dissimilar were the largest. Moreover, the dendrogram suggests that all sand gobies over 11 cm in total length are very different from those below that size, i.e. the most similar size classes are the smallest ones.

Table 5. Manhattan distances between sheared score centroids of *Neogobius fluviatilis* size classes.

Size class	<10 cm	10-11 cm	11-12 cm	>12 cm
<10 cm	0.000			
10-11 cm	1.802	0.000		
11-12 cm	4.845	3.043	0.000	
>12 cm	9.009	7.207	4.164	0.000

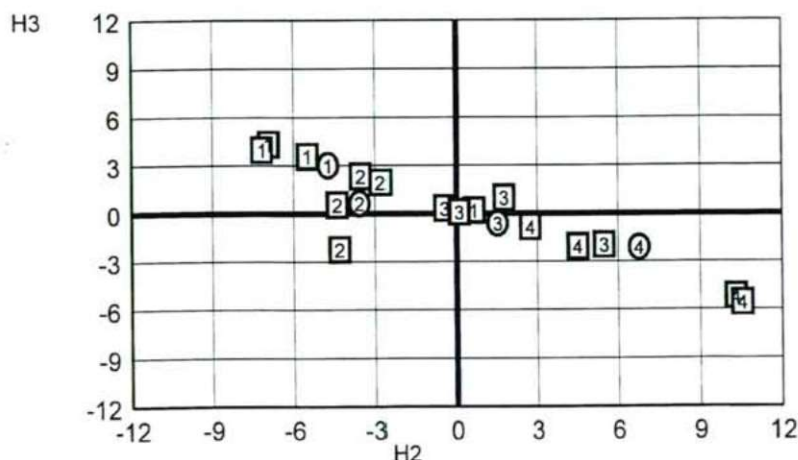


Fig. 4. Sheared principal component (H2 and H3) scores of sand goby specimens (boxes) of particular size classes (1-4) and centroids for size classes (circles).

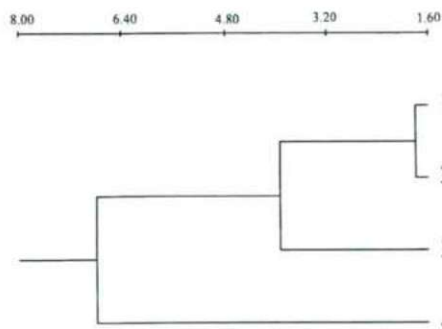


Fig. 5. Dendrogram of size class (1-4) centroids of *Neogobius fluviatilis*.

## Discussion

The total lengths of the specimens examined in the paper of VASILEVA (1988) and in the present work indicated that the samples were very similar and thus might be comparable. No data on the distribution of the particular size classes were given in the work of VASILEVA (1988). It must be taken into account that this work included a few large specimens (i.e. four over 12 cm in total length), and this might be the reason for the differences between particular populations that appeared in some features. The description of particular skull bones mostly corresponds to that of VASILEVA (1988). Similar prominent features regarding both visual impression and descriptive statistics of the whole skull and particular bones (i.e. dental, articulare, hyomandibulare and cleithrum) and the ratios of some bone parts were also stressed here. It was suggested that there was no great variability of these traits. The comparison between sand goby populations from the River Sava and the Caspian Sea tributaries and the only specimen from the Azov Sea revealed that the sand gobies were rather similar in many characters. Many differences in examined characters were not significant on ANOVA, and even on pairwise testing, i.e. the approximative pairwise comparison ( $M_{ij} \pm 2s_{ij}$ ). However, it seems that the sand gobies from the River Sava are more similar to those from the Caspian Sea tributaries in certain traits than to the specimen from the Azov Sea, and in other traits they are different. This strongly corroborated an inference on the consistent range of variability of this species (VASILEVA, 1988).

The intrapopulation variability between the particular size classes was noteworthy. Although several characters varied significantly, especially those in the largest sand gobies (over 12 cm in total length), they had no outstanding impact on their ontogenetic trajectories, most probably due to the small variability inherent to them. The UPGMA clustering of size class centroids (Fig. 5) and the concordance with the ontogenetic trajectories corroborates an inference on the direct pattern of morphology formation through the examined size classes.

With regard to the facts that both *Impter* almost exclusively loaded PC1, and the sheared shape axes (i.e. H2 and H3) did not differ from their precursors (PC2 and PC3), it was evident that the effect of size on the overall morphology of the sand goby was extremely small. This was probably because of the size range of the analyzed specimens. *Impter* could be regarded as an extraordinary variable trait, since it had a variation coefficient of 63.1% (Table 1). Its correlation with size seemed remarkable, although descriptive statistics results and ANOVA testing (Tables 1 and 2) did not reveal this character as significant and discriminative for size classes, implying that it varied individually and stochastically. It is possible that the relative small *Impter* in the 10-10.9 cm size class affected the multivariate analysis strongly by its variation. Thus, this character may be regarded as inappropriate for any discrimination analysis.

The most variable traits related to the jaw apparatus of the splanchnocranium (both dermal and substituent), and to the gill cover. Skull elements (i.e. sphenotic and pterotic) that changed significantly in particular size classes did not participate

markedly in the overall variation, and thus did not load the first three principal components.

Only one character of 8 reported as noteworthy for variation due to size in sand gobies from the Caspian Sea watershed (VASILEVA, 1988), i.e. Spt, varied similarly as in sand gobies from the River Sava according to unifactorial ANOVA testing. Two characters, Lprocsph and hartic, were prominent by unifactorial ANOVA and PCA, which implies that they are both rather variable and at the same time discriminative for size classes. Thus, it seems that they should be taken into account for further phylogenetic considerations.

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## AUCHENORRHYNCHA ASSEMBLAGES OF THE "ÁSOTTHALMI LÁPRÉT" NATURE CONSERVATION AREA IN HUNGARY I.

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(Received: May 30, 1996)

### Abstract

The basic data evaluation of the Auchenorrhyncha fauna of the "Ásotthalmi Láprét" grassland area located in the southern part of Hungary was based on samples taken in 1990. The number of samples taken was 2070 (Barber traps) and 1242 (platter traps). According to the investigations made so far, 101 Auchenorrhyncha species live in the area. The number of species was higher here than in dry sandy grasslands or in wet areas, because of the high degree of heteromorphy and different microclimatic conditions. On the basis of Schiemenz's system, *Euscelis incisus* and *Turrutus socialis* were the dominant species, and *Anaceratagallia ribauti*, *Graphocraerus ventralis* and *Psammotettix kolosvarensis* were the subdominant species. More than fifty percent of the species were characteristic to xerotherm habitats. Most of the species spent the winter in the form of eggs. The northern distribution border of eleven species, and the western distribution border of four species, was the Carpathian Basin.

*Key words:* Auchenorrhyncha, fauna, bionomics, ecological valence.

### Introduction

The protection of natural and semi-natural biotops was mostly based on botanical values. However, after this, a wider scope of undiscovered values would still be needed. Hopefully, these yet undetermined values could spread to other groups of living organisms. As a part of a similar research project, this paper was trying to discover the Auchenorrhyncha fauna of the grassland area which only recently became protected. Research like this has mostly been made about the Great Plains region (KOPPÁNYI and WOLCSÁNSZKY, 1955; GYÖRFFY, 1980, 1982; OROSZ, 1981; GYÖRFFY and KINCSEK, 1986).

## Materials and methods

### *The examined area*

The "Ásotthalmi Láprét" area, situated in the southern part of Csongrád county, between the rivers Danube and Tisza (Fig. 1), had an area of ninety-five ha. This landscape appeared heteromorphous because of the varied relief with sand hills and wind furrows. The maximum height difference approximated 4-5 m.

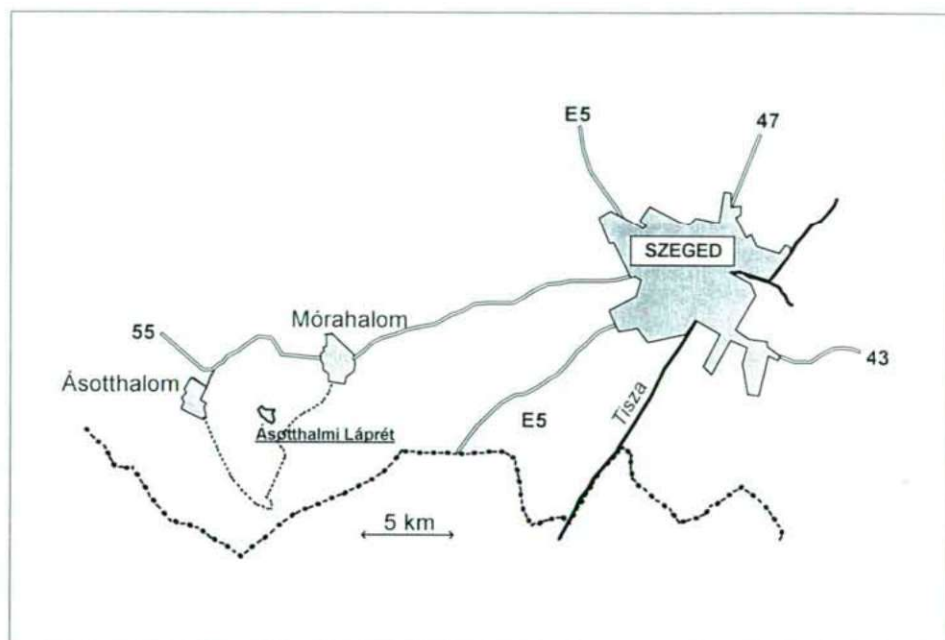


Fig. 1. The location of the 'Ásotthalmi Láprét' in the southern part of the Great Hungarian Plain.

The ten-to-twenty meter deep quicksand level of the table-land between the Rivers Danube and Tisza used mainly to be moved by the northwest winds of April. As a result of this, the previously mentioned sandhills and windfurrows were created. In these windfurrows, holocen-time water was collected which formed shallow ponds. These former ponds were fed by both rain and the dripping ground water which was rich in salts. As a result of the plants and the strong summer evaporation, an alkaline solution highly concentrated in salts was created. There used to be a sodic pond of this kind in most of the "Ásotthalmi Láprét". In the 1960s, it was drained with the help of a channel put in the middle of the pond. As a result of this, in the deeper parts marsh meadows appeared.

According to soil research drillings, under 90-180 cm of fine sand mixed with tiny sand could be found. Above this level, there was a 60-120 cm deep carbonate mud, which was covered by 30-60 cm deep quicksand. This was made of tiny sand mixed with fine sand. The upper sixty cm was soil-like and rich in humus. Under this level, the grey or white color was the result from leaching. The carbonate concentration of the carbonate mud was very high, as it reached seventy percent. The decreasing of the carbonate concentration of the northern sample at 130-150 cm was the same as the increasing of the sand fraction ratio. This sand fraction ratio resulted from sand carried by the wind from the higher edges of the terrain.

### *Botanical characterization*

There were five characteristic plant associations in the area. On the highest terrain *Astragalus-Festucetum rupicolae* was found. Lower from that, *Chrysopogono-Caricetum humilis* appeared. In the wind



furrows, both *Salicetum rosmarinifoliae*, and *Succiso-Molinietum coeruleae* were developed. Finally, on the lowest terrain, *Scirpo-Phragmitetum* could be found (FÜZNE, 1989; GALUSKA, 1992).

Twenty-nine percent of the plant species indicated degradation, but the proportion of weeds was only 4.6 p.c. The reason for this low rate was the given treatment for prevention of the expansion of reeds. Mowing has been carried out in the last thirty years, usually in July. In 1990 this happened in two parts, first during the last week of July, and second at the end of August.

### *Collecting methods*

Investigations were carried out in 1990, from 9 March till 30 November, on the northern part of the area. Barber and platter traps were used. The traps were arranged in nets; 115 Barber traps in five columns and twenty-three rows, and sixty-nine platter traps in three columns and twenty-three rows, respectively. The Barber traps were five meters apart from each other, and the platter traps were ten meters apart from each other. The trap-net covered a plot of 122 m long and 36 m broad. The numbers of rows increased from the highest to the lowest terrain. Samples were taken bi-weekly (Table 1). Less samples were available for platter traps because these traps were sometimes destroyed by mowing. The number of samples taken were 2070 (Barber traps) and 1242 (platter traps), for a combined total of 3312.

## **Results and discussion**

Altogether 44946 specimens were trapped (5212 larvae and 5528 adults by Barber traps, and 11890 larvae and 21816 adults by platter traps). These belonged to 101 species (in Barber traps 80 species, in platter traps 90 species). The number of species was higher here, than in dry sandy grasslands or in wet areas, because of the high degree of heteromorphy and different microclimatic conditions. For example, in another sandy grassland in Hungary,  $31.25 \pm 1.78$  species were collected in sand hills (over an average of four years). Also,  $41.5 \pm 3.64$  species were collected in windfurrows by sucking apparatus. Forty-six and forty-one species were trapped on the sand hills and twenty-three and twenty-nine species in the windfurrows by Barber traps during two years (GYÖRFFY, 1982). At three sodic zonations differing from each other in 10-15 cm ground level heights, fifty-one Auchenorrhyncha species were collected in Hungary during two years (GYÖRFFY and KINCSEK, 1986). During the same time, sixty-six species occurred in four plant associations of a grassland — sand forest mosaic habitat (GYÖRFFY and KINCSEK, 1988). In low-productive, non-cultivated meadow moors (in Poland), the species numbers were thirty-five and twenty-three respectively (ANDRZEJEWSKA, 1965). From four types of upland seeded pastures in Kansas,  $21.13 \pm 4.27$  species were collected between 1965 and 1968 (BLOCKER *et al.*, 1972). On average,  $24.88 \pm 6.31$  species were found by SCHIEMENZ in eight dry and half-dry grassland types of Middle-Europe (SCHIEMENZ, 1969). The Auchenorrhyncha faunas of twelve moors and bogs in Germany consisted of  $23.08 \pm 7.11$  species (SCHIEMENZ, 1976), of seven bogs in Thuringer Wald and in Harz,  $25.28 \pm 7.59$  species (SCHIEMENZ, 1975) and of ten German moss-moors  $41.4 \pm 9.15$  species (SCHIEMENZ, 1971) respectively.

Table 1. Sampling periods and methods.

	Time period	Barber trap	Platter trap
1	03.09-03.23	X	X
2	03.23-04.06	X	X
3	04.06-04.20	X	X
4	04.20-05.04	X	
5	05.04-05.18	X	X
6	05.18-06.01	X	X
7	06.01-06.15	X	X
8	06.15-06.29	X	
9	06.29-07.13	X	X
10	07.13-07.27	X	
11	07.27-08.13	X	
12	08.13-08.24	X	
13	08.24-09.07	X	X
14	09.07-09.21	X	X
15	09.21-10.05	X	X
16	10.05-10.19	X	
17	10.19-11.02	X	
18	11.02-11.30	X	

The list of species, number of specimens, and percent of dominance can be found in Table 2. For evaluating the dominance relations, we used SCHIEMENZ'S division, (SCHIEMENZ, 1969) according to which (D-dominance):

D-group	D per cent	
5	64 - 100	3, 4, 5: dominant species
4	36 - 64	2: subdominant species
3	16 - 36	+, 1: accessory species
2	4 - 16	
1	1 - 4	
+	<1	

There were some different divisions in the literature, but we did not know objective criteria for limitations of dominance classes (RAATIKAINEN and VASARAINEN, 1976; GASTON, 1994). We chose the SCHIEMENZ'S system due to its better comparison of data. On the basis of this, *Euscelis incisus* and *Turrutus socialis* were the dominant species, and *Anaceratagallia ribauti*, *Graphocraerus ventralis* and *Psammotettix kolosvarensis* were the subdominant species (Table 2.).

On the basis of the division of the occurring species according to ecological valence, more than 50 percent of the species live in xerotherm habitat (Fig. 2).

Comparing these values with SCHIEMENZ'S data (Table 3), it can be noted that 32.32 percent of the species in this area could not be found in SCHIEMENZ'S categories. The value of stenotop species living in xerothermous biotopes (X) was especially lower in the investigated area.

Table 2. List of species, number of individuals, percent of dominance (D.p.c.), and dominance classes according to SCHIEMENZ (d.c.). The abbreviations will be used in the following paper.

Species	abbrevi- ation	Platter trap	Barber trap	Sum.	D.p.c.	d.c.
<i>Acanthodelphax spinosus</i> (FIEBER, 1866)	Aca.spi.	5	5	10	0.037	+
<i>Adarrus notatifrons</i> (KIRSCHBAUM, 1868)	Ada.not.	323	68	391	1.430	1
<i>Agallia laevis</i> RIBAUT, 1935	Aga.lae.	217	48	265	0.969	+
<i>Alebra wahlbergi</i> (BOHEMAN, 1845)	Ale.wah.	1	0	1	0.004	+
<i>Anaceratagallia ribauti</i> (OSSIANNILSSON, 1938)	Ana.rib.	555	661	1216	4.447	2
<i>Anakelisia perspicillata</i> (BOHEMAN, 1845)	Ana.per.	11	2	13	0.048	+
<i>Anoscopus serratae</i> (FABRICIUS, 1775)	Ano.ser.	7	6	13	0.048	+
<i>Aphrodes albiger</i> (GERMAR, 1821)	Aph.alb.	13	27	40	0.146	+
<i>Aphrodes bicinctus</i> (SCHRANK, 1776)	Aph.bic.	409	209	618	2.260	1
<i>Arocephalus languidus</i> (FLOR, 1861)	Aro.lan.	716	246	962	3.518	1
<i>Arthaldeus pascuellus</i> (FALLÉN, 1826)	Art.pas.	5	3	8	0.029	+
<i>Arthaldeus striifrons</i> (KIRSCHBAUM, 1868)	Art.str.	288	5	293	1.072	1
<i>Artianus interstitialis</i> (GERMAR, 1821)	Art.int.	225	32	257	0.940	+
<i>Athysanus argentarius</i> METCALF, 1950	Ath.arg.	34	16	50	0.183	+
<i>Austroagallia sinuata</i> (MULSANT et REY, 1855)	Aus.sin.	124	8	132	0.483	+
<i>Bobacella corvina</i> (HORVÁTH, 1903)	Bob.cor.	179	44	223	0.816	+
<i>Chlorita dumosa</i> (RIBAUT, 1933)	Chl.dum.	18	31	49	0.179	+
<i>Chlorita paolii</i> (OSSIANNILSSON, 1939)	Chl.pao.	26	5	31	0.113	+
<i>Cicadella viridis</i> (LINNAEUS, 1758)	Cic.vir.	36	2	38	0.139	+
<i>Cicadula persimilis</i> (EDWARDS, 1920)	Cic.per.	11	2	13	0.048	+
<i>Cixius simplex</i> (HERRICH-SCHÄFFER, 1835)	Cix.sim.	1	0	1	0.004	+
<i>Conosanus obsoletus</i> (KIRSCHBAUM, 1858)	Con.obs.	93	12	105	0.384	+
<i>Delphacodes capnodes</i> (SCOTT, 1870)	Del.cap.	2	0	2	0.007	+
<i>Delphacodes venosus</i> (GERMAR, 1830)	Del.ven.	0	1	1	0.004	+
<i>Dicranotropis hamata</i> (BOHEMAN, 1847)	Dic.ham.	2	2	4	0.015	+
<i>Dictyophara pannonica</i> (GERMAR, 1830)	Dic.pan.	0	3	3	0.011	+
<i>Diplocolenus abdominalis</i> (FABRICIUS, 1803)	Dip.abd.	1	0	1	0.004	+
<i>Doratura heterophylla</i> HORVÁTH, 1903	Dor.het.	135	13	148	0.541	+
<i>Doratura homophylla</i> (FLOR, 1861)	Dor.hom.	498	59	557	2.037	1
<i>Doratura impudica</i> HORVÁTH, 1897	Dor.imp.	174	55	229	0.837	+
<i>Enantiocephalus cornutus</i> (HERRICH-SCHÄFFER, 1838)	Ena.cor.	1	0	1	0.004	+
<i>Eupelix cuspidata</i> (FABRICIUS, 1775)	Eup.cus.	358	50	408	1.492	1
<i>Eupteryx aurata</i> (LINNAEUS, 1758)	Eup.aur.	5	4	9	0.033	+
<i>Eupteryx notata</i> CURTIS, 1837	Eup.not.	223	115	338	1.236	1
<i>Eupteryx stachydearum</i> (HARDY, 1850)	Eup.sta.	1	0	1	0.004	+
<i>Eurybregma nigrolineata</i> SCOTT, 1875	Eur.nig.	4	0	4	0.015	+
<i>Euryula lurida</i> (FIEBER, 1866)	Eur.lur.	0	2	2	0.007	+
<i>Euscelis incisus</i> (KIRSCHBAUM, 1858)	Eus.inc.	5611	946	6557	23.980	3
<i>Evacanthus acuminatus</i> (FABRICIUS, 1794)	Eva.acu.	1	0	1	0.004	+
<i>Forcipata citrinella</i> (ZETTERSTEDT, 1828)	For.cit.	63	7	70	0.256	+
<i>Forcipata forcipata</i> (FLOR, 1861)	For.for.	0	1	1	0.004	+
<i>Graphocera ventralis</i> (FALLÉN, 1806)	Gra.ven.	1222	191	1413	5.167	2
<i>Gravesteiniella boldi</i> (SCOTT, 1870)	Gra.bol.	16	2	18	0.066	+
<i>Hecalus glaucescens</i> (FIEBER, 1866)	Hec.gla.	2	1	3	0.011	+
<i>Hephathus nanus</i> (HERRICH-SCHÄFFER, 1835)	Hep.nan.	339	67	406	1.485	1
<i>Idiocerus</i> sp.	Idi. sp.	1	0	1	0.004	+
<i>Jassargus obtusivalvis</i> (KIRSCHBAUM, 1868)	Jas.obt.	0	3	3	0.011	+
<i>Jassargus sursumflexus</i> (THEN, 1902)	Jas.sur.	479	84	563	2.059	1
<i>Jassidaeus lugubris</i> (SIGNORET, 1865)	Jas.lug.	8	4	12	0.044	+
<i>Kelisia brucki</i> FIEBER, 1878	Kel.bru.	0	4	4	0.015	+
<i>Kelisia guttula</i> (GERMAR, 1818)	Kel.gut.	74	12	86	0.315	+
<i>Kelisia pallidula</i> (BOHLMAN, 1847)	Kel.pal.	48	11	59	0.216	+



Table 2. (continued)

Species	abbrevi- ation	Platter trap	Barber trap	Sum.	D.p.c.	d.c.
<i>Kelisia perrieri</i> RIBAUT, 1934	Kel.per.	16	6	22	0.080	+
<i>Kelisia praecox</i> HAUT, 1935	Kel.pra.	1	0	1	0.004	+
<i>Kelisia vittipennis</i> (J. SAHLBERG, 1868)	Kel.vit.	1	0	1	0.004	+
<i>Laodelphax striatellus</i> (FALLÉN, 1826)	Lao.str.	4	4	8	0.029	+
<i>Lepyronia coleoptrata</i> (LINNAEUS, 1758)	Lep.col.	217	60	277	1.013	1
<i>Macrostelus fieberi</i> (EDWARDS, 1889)	Mac.fie.	1	1	2	0.007	+
<i>Megophthalmus scanicus</i> (FALLÉN, 1806)	Meg.sca.	48	56	104	0.380	+
<i>Mendraus paucillius</i> (FIBER, 1869)	Men.pau.	25	10	35	0.128	+
<i>Micantulina stigmatipennis</i> (MULSANT et REY, 1855)	Mic.sti.	13	0	13	0.048	+
<i>Mocuellus collinus</i> (BOHEMAN, 1850)	Moc.col.	380	77	457	1.671	1
<i>Mocydia crocea</i> (HERRICH-SCHÄFFER, 1837)	Moc.cro.	5	1	6	0.022	+
<i>Mocydiopsis attenuata</i> (GERMAR, 1821)	Moc.att.	0	1	1	0.004	+
<i>Mocydiopsis parvicauda</i> RIBAUT, 1939	Moc.par.	84	3	87	0.318	+
<i>Muellerianella extrusa</i> SCOTT, 1871	Mue.ext.	0	5	5	0.018	+
<i>Muellerianella</i> sp.	Mue.sp.	29	0	29	0.106	+
<i>Muirodelphax aubei</i> (PERRIS, 1857)	Mui.aub.	9	7	16	0.059	+
<i>Neocalitrus fenestratus</i> (HERRICH- SCHÄFFER, 1834)	Neo.fen.	195	63	258	0.944	+
<i>Neophilaenus campestris</i> (FALLÉN, 1805)	Neo.cam.	48	17	65	0.238	+
<i>Neophilaenus lineatus</i> (LINNAEUS, 1758)	Neo.lin.	29	2	31	0.113	+
<i>Neophilaenus minor</i> (KIRSCHBAUM, 1868)	Neo.min.	0	5	5	0.018	+
<i>Ommatidiotus dissimilis</i> (FALLÉN, 1806)	Omm.dis.	4	1	5	0.018	+
<i>Paluda preyssleri</i> (HERRICH-SCHÄFFER, 1838)	Pal.pre.	1	0	1	0.004	+
<i>Paluda vitripennis</i> (FLOR, 1861)	Pal.vit.	357	57	414	1.514	1
<i>Paralimnus</i> sp.	Par.sp.	10	1	11	0.040	+
<i>Philaenus spumarius</i> (LINNAEUS, 1758)	Phi.spu.	132	45	177	0.647	+
<i>Psammotettix alienus</i> (DAHLBOM, 1850)	Psa.ali.	154	3	157	0.574	+
<i>Psammotettix cephalotes</i> (HERRICH- SCHÄFFER, 1834)	Psa.cep.	8	7	15	0.055	+
<i>Psammotettix confinis</i> (DAHLBOM, 1850)	Psa.con.	26	1	27	0.099	+
<i>Psammotettix kolosvarensis</i> (MATSUMURA, 1908)	Psa.kol.	1173	184	1357	4.963	2
<i>Psammotettix provincialis</i> (RIBAUT, 1925)	Psa.pro.	13	3	16	0.059	+
<i>Psammotettix slovacus</i> DLABOLA, 1948	Psa.slo.	2	0	2	0.007	+
<i>Recilia schmidtgeni</i> (WAGNER, 1939)	Rec.sch.	791	24	815	2.981	1
<i>Ribautodelphax albostratus</i> (FIEBER, 1866)	Rib.alb.	32	26	58	0.212	+
<i>Ribautodelphax imitans</i> (RIBAUT, 1953)	Rib.imi.	130	61	191	0.699	+
<i>Streptanus aemulans</i> (KIRSCHBAUM, 1868)	Str.aem.	151	61	212	0.775	+
<i>Stroggylocephalus agrestis</i> (FALLÉN, 1806)	Str.agr.	5	0	5	0.018	+
<i>Struebingianella palliceps</i> (HORVÁTH, 1897)	Str.pal.	281	226	507	1.854	1
<i>Tetartostylus pellucidus</i> WAGNER, 1951	Tet.pel.	15	3	18	0.066	+
<i>Tettigometra atra</i> HAGENBACH, 1825	Tet.atr.	1	0	1	0.004	+
<i>Tettigometra fusca</i> FIEBER, 1865	Tet.fus.	2	0	2	0.007	+
<i>Tettigometra impressopunctata</i> DUFOR, 1846	Tet.imp.	18	4	22	0.080	+
<i>Tettigometra obliqua</i> (PANZER, 1799)	Tet.obl.	0	3	3	0.011	+
<i>Toya minuscula</i> (HORVÁTH, 1897)	Toy.min.	7	4	11	0.040	+
<i>Toya propinqua</i> (FIBER, 1866)	Toy.pro.	39	0	39	0.143	+
<i>Trypetimorpha fenestrata</i> A.COSTA, 1862	Try.fen.	0	1	1	0.004	+
<i>Turrutus socialis</i> (FLOR, 1861)	Tur.soc.	4466	940	5406	19.770	3
<i>Ulopa trivialis</i> GERMAR, 1821	Ulo.tri.	112	410	522	1.909	1
<i>Xanthodelphax stramineus</i> (STAL, 1858)	Xan.str.	2	1	3	0.011	+
<i>Zyginidia pullula</i> (BOHEMAN, 1845)	Zyg.pul.	214	75	289	1.057	1

Table 3. Comparison of Auchenorrhyncha assemblages of dry grasslands (SCHIEMENZ, 1969) and of the investigated area according to their ecological valence.

ecological valence	Percent of all species	
	SCHIEMENZ' data	Own data
(X)	44.07	31.25
(X-m),(X-m-h)	24.33	16.66
(x-m),(x-m-h)	21.71	11.45
(x-M-h),(x-M-H),(x-m-H)	9.85	8.32
without X	-	32.32

Most of the species spend the winter in the form of eggs (Fig. 3). The percentage of specimens under the class "unknown" was only 1.89 %.

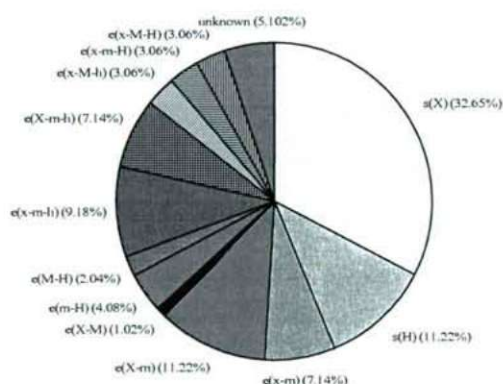


Fig 2. Division of Auchenorrhyncha species according to their ecological valence (e: eurytopic; s: stenotopic; x: xerophilous; m: mesophilous; h: hygrophilous; the capital letters indicate stronger dominances)

Table 4. Comparison of bionomical data (wintering stage, number of generations per year) of the occurring species in the "Ásotthalmi Láprét" with similar data from moors and dry grasslands (SCHIEMENZ, 1971)

wintering stage	moors			dry grasslands			Ásotthalmi Láprét			
	1 gen.	2 gen.	Sum.	1 gen.	2 gen.	Sum.	1 gen.	2 gen.	3 gen.	Sum.
adult	9.6	0	9.6	12.5	6.6	19.1	11.22	3.06	0	14.28
larvae	23.1	3.8	26.9	2.9	7.4	10.3	3.06	9.18	0	12.24
egg	50.0	13.5	63.5	33.8	36.8	70.6	30.61	29.59	2.04	62.24
Sum.	82.7	17.3	100	49.2	50.8	100	44.89	41.83	2.04	88.76
							unknown: 11.22%			

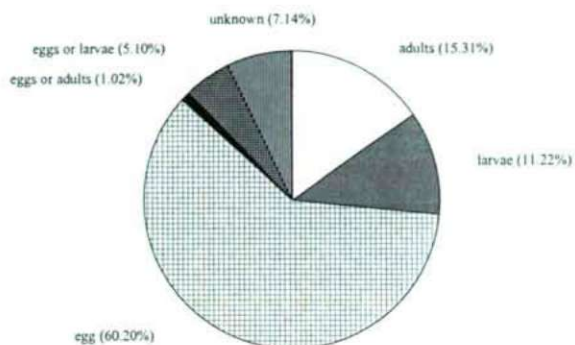


Fig. 3. Division of Auchenorrhyncha species according to wintering stage

Most species had one or two yearly generations (Fig. 4). The percentage of specimens under the class "unknown" was only 1.91%.

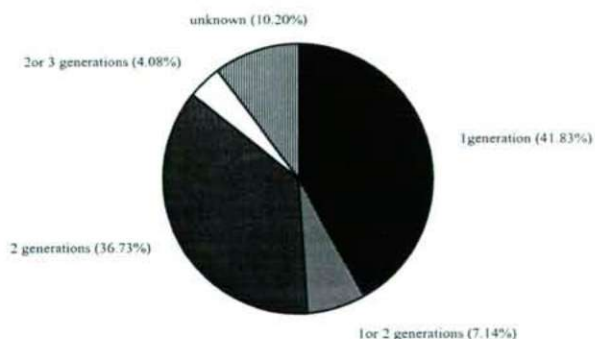


Fig. 4. Division of Auchenorrhyncha species according to the number of generations in a year

We compared this acquired data with similar data from moors and dry grasslands (SCHIEMENZ, 1971). On the basis of this data comparison, the investigated area was closer to the dry grasslands (Table 4).

The species occurring in the "Ásotthalmi Lápért" area were widely spread (holarctic, palearctic, and western-palearctic), but approximately twelve percent of the species were more common in the southern areas. The northern distribution border of eleven species, and the western distribution border of four species, was the Carpathian Basin.



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## POSSIBILITIES AND IMPORTANCE OF HUMAN MEIOTIC STUDIES

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(Received: October 31, 1996)

### Abstract

In order to understand the mechanism by which various chromosomal abnormalities are brought about, the study of meiotic process is necessary.

The study of spermiogenesis is a rather neglected field although meiotic errors may lead to infertility. Appropriate methods are available to study meiosis by which chromosomal preparations can be produced from the testicular tissue, from mature sperms and ova to reveal chromosomal anomalies.

The most common alterations in the meiotic process are translocations. These can be traced back to breaks and abnormal chromosomal pairing.

The mechanisms of autosomal and sex-chromosomal alterations with regard to their formation can be distinguished. In some cases the study of meiosis can give a clue regarding the cause of aneuploidies.

It seems to be of special importance whether the aneuploidy is produced in the first or the second cleavage of the meiotic process.

*Key words:* meiosis, spermiogenesis, genetics, non-disjunction, translocation

### Introduction

Study into the human meiosis is an important, but hitherto relatively neglected field of genetics. The importance of such studies are given by the fact that failures in the meiotic process may lead to various abnormalities in the fetus.

The main details of the meiotic process are well known. Our own contribution to the meiotic process were to reveal the possibilities to model the production of chromosomal abnormalities that are known in the human being — in experimental animals (SZEMERE and ZSIBRITA, 1978; YANAGIMACHI, 1976).

There have been virtually no meiotic studies so far. The reason for that is that meiotic studies are more difficult to carry out than those from lymphocytes, amniotic cells or somatic samples. But still, in order to get to a better understanding of the mechanism behind the chromosomal aberrations, deeper insight into meiotic process is of utmost importance (BIGGERS et al., 1971; CHANDLY, 1981; MARTIN, 1980).



## Material and method

The human semen sample is collected in a sterile container and processed as soon as liquefaction has occurred (10 to 20 min at 37 °C). Total sperm count, number of sperm/ml, motility, forward progression, the ratio of live to dead sperm, and the morphology of the sperm are determined on a small portion of the sample. Approximately 10 ml of BWW working pollution at 37 °C is added to the semen. The semen is centrifuged at 600 g for 6 min, the supernatant is decanted, and the pellet is resuspended in 10 ml BWB. The sperm are washed two more times. The final pellet is resuspended in 5 ml of BWB solution.

The resuspended sample is put into a thermostat of 37 °C for 18 hours. The sediment is put into 0.1% trypsin for two minutes. The sample is hypotonised in KCL and dropped onto warmed slides. After two days the specimens are stained and examined under the light microscope.

## Results

### *Human meiosis*

The meiotic process differs in the males and in the females. In females it starts during embryonic development and the eggs mature in a cyclic way from puberty, while in the male the production of germ cells is continuous.

Meiosis starts from diploid (2n) stem cells in each case, and — as a result — haploid (n) mature germ cells are produced. The haploid chromosomal number is the consequence of two subsequent divisions during meiosis. One of the most striking difference from mitosis is that the prophase meiosis I is carried out in four stages: leptotene, zygotene, pachytene and diplotene. After the first division haploid cells of two chromatids are produced when the chromosomes of maternal and paternal origin are randomly distributed in the daughter-cells.

The first meiotic division is followed by an extremely short interphase in which there is no DNA replication, thus leading to the production of haploid germ cells in meiosis II, because in this process the chromatids of the two chromosomes are separated, thus producing four germ cells.

The importance of meiosis is that it ensures the maximal conservatism, i.e. the maintenance of the chromosome number characteristic of the species on the one hand and through recombination to genetic variability, on the other.

Eventual meiotic errors may lead to serious anomalies in the offspring. In this respect the most striking phenomenon is non-disjunction that brings about trisomies and nullisomies. Non-disjunction may occur both in meiosis I and meiosis II (SZEMERE and CHANDLY, 1976).

### *Examination of the meiotic process*

There has been a breakthrough in the human and mammal meiotic studies from 1964, when the air-drying method of the spermatocytes had been introduced. This was followed by combination with other specific techniques, like G-banding, fluorescent techniques, etc. A new era has appeared in the field of the investigation of meiotic chro-

mosomes when COUNCE and MEYER, (EVANS et al., 1964; MARTIN, 1980) introduced the quick and simple 'micro-spreading' technique in 1973. The method had been improved by (MARTIN, 1963). These methods made the investigation of specific proteins that are produced in different stages of prophase, like e.g. the study of the synaptonemal complex possible. These proteins join the homologous chromosomes together during the pairing in the zygotene prophase meiosis I, through pachytene towards the diplotene, when the separation of the bivalents begin. The full separation of the chromosomes ends in diakinesis.

Study of meiotic process may occur from preparations of testicular material, mature sperm, ovaries and mature eggs.

#### *Preparation of human sperm*

Maturation of sperm is continuous in men from puberty, thus meiotic chromosomes can be analysed after the preparation procedure. The methodology had been described by BIGGERS and MARTIN (1971, 1988) and modified by ourselves (MARTIN, 1963).

#### *Detection of translocations and non-disjunctions in the meiotic process*

What are the factors that indicate the meiotic studies ?

In cases of healthy, fertile men virtually no biopsies are taken, thus the main source of studying the meiotic process is the clinical study of infertile men. The indication of the cytogenetic study of meiosis is mainly oligospermy or azoospermy. Infertility is often caused by chromosomal abnormality that can be proved by cytogenetic analysis. Infertile men are screened world-wide and cytogenetic studies on the germ-cells should be part of this screening. The target is to fully reveal the disturbances of the genome thus ensuring the identification of the spermatogenetic block in the process of spermiogenesis (AURIAS and BALKAN, 1978, 1983).

Damage of the germ-cells, lack or failure of chromosome pairing as well as translocation phenomena are connected with one another (ROBEZ, 1986; HASSOLD and MATSUYAMA, 1979).

Failures may occur both in spermiogenesis and oogenesis (CHANDLY, 1976; FOREJT, 1981; MOSES, 1975).

Abnormalities during the process of germ-cell production appear in a different way in male and female offspring. In women the heterozygote carrier status does not necessarily prevent fertility, thus damage in the process of oogenesis may remain without consequences.

There is also a remarkable difference in the maturation mechanism of spermiogenesis and oogenesis. While — according to the widely accepted hypothesis — the XX bivalents are active during prophase meiosis I, the XY bivalents are not.

There is a tendency in the oocytes to failure or lack of chromosome pairing, which is explained by the transcriptional activity of the X-chromosomes (SANGER, 1971, 1977). It is highly probable that oocytes with a mispairing of chromosomes cannot mature in the adult ovaries and this is also the factor leading to the atresia of the



follicles during embryonic development. Damage in the process of spermiogenesis can be explained, among others, by the breakage of the distal part of the long arm of the Y chromosome, since the gene controlling spermiogenesis can be found in this region. Earlier, this gene was thought to be the TDF (Testicular Determining Factor), but it turned out that there are two separate genes in question. According to our present knowledge, TDF can be found in the short arm of the Y chromosome in a "pseudosomal" domain. The spermiogenesis controlling gene is responsible - beside the formation of germ cells - for the migration of them, thus the failure of this gene may also lead to migration disturbances.

Study of meiotic processes may give an explanation of the causes of the most striking abnormalities, like aneuploidies and translocations.

It has been shown by RUSSEL and MONTGOMERY in 1974 that different types of aneuploid fetuses are born if non-disjunction of the chromosomes occurs in the first or in the second meiotic division of the spermiogenesis or oogenesis. This conclusion has also been drawn by SANGER (1971, 1977), LAURITSEN and FRIDRICH (1976), NIKAWA (1977), HASSOLD (1980) as well as RACE and SANGER (1969, 1977). According to their studies, meiotic non-disjunction may affect both the somatic and the sex-chromosomes.

Phenomena described above indicate that meiosis may be damaged both in the maternal and paternal organism. Both may lead to infertility, this is why the study of meiotic processes is so important.

In our future studies we would like to concentrate on the study of spermiogenesis, thus making a contribution to the prevention of the consequences caused by infertility.

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## CHARACTERISTIC PARAMETERS OF HEAD MEASUREMENTS IN HUNGARIAN CHILDREN AGED 3-18 YEARS

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(Received: October 1, 1996)

### Abstract

The authors calculated the percentile curves of six head measurements and three head indices for Hungarian boys and girls from the data reported earlier (FARKAS and NYILAS, 1988).

*Key words:* percentile curves, head measurements, Hungarian boys and girls.

### Introduction

Biological anthropology is a typically interdisciplinary science. It is necessary to stress the use of the adjective biological here for there are nowadays many types of anthropology. Besides the earlier philosophical, social and cultural forms of anthropology, for instance, we can currently also speak of instrumental anthropology and visual anthropology. Biological anthropology studies the biological feature of man.

Man is a biological and social creature. Accordingly, the anthropologist or human biologist who studies the biological features of man must collaborate with experts in many other branches of science in order to solve the problems that arise in the course of the research.

One branch of science that is particularly important from this respect is odontology. Throughout the history of Hungarian anthropology, during a period of more than a hundred years, there have been numerous cases when an anthropologist and an odontologist have worked together to solve some scientific problem. Such joint activity has been performed in part during investigations on findings originating from examinations on the living population.

The collaboration has always been close and fruitful between the Department of Anthropology at József Attila University and the Dental and Oral Surgical Clinic at Albert Szent-Györgyi Medical University in Szeged. This collaboration was initiated by Professors DEZSŐ HATTYASI and LAJOS BARTUCZ, was further developed by Professors KÁROLY TÓTH and PÁL LIPTÁK, and is continuing present. The effectiveness of this



joint work is illustrated by theses that have been written in connection with the award of a number of high level scientific degrees.

### Background

The question may justifiably be posed of why cooperation yielding such useful results should develop between odontologists and anthropologists.

The physician nearly always deals with individual patients and only rarely has a possibility to examine many thousands of individuals from one particular aspect. If the odontologist wishes to study the variations in dental enamel, for example, it is scarcely possible to extract the healthy teeth of several hundred humans, examine them and replace them. In contrast, this does not constitute a problem as concerns material originating from excavations.

Anthropologists may observe a great number of things, but as they are biologists they do not possess adequate medical knowledge and hence may have difficulty in interpreting a given phenomenon. Good collaboration between odontologists and anthropologists, however may overcome such difficulties.

Thus, when the two institutions are denoted in the name of the conference "Tooth regulation and Anthropology", this is not by chance. It may be stated with confidence that the collaboration is based on a friendly and scientific connection dating back several decades, and we hope that it will continue to be productive in the future.

The work of the anthropologist is particularly useful, for instance, if he or she can construct tables based on many thousands of measured data relating to the development of children, and if these tables can be utilised in medical practice.

In this respect, a good connection has developed primarily with paediatricians. In the course of their everyday work, paediatricians already apply development tables serving for control of body growth, based on parameters relating to height, weight and chest measurements. This in itself is important, for in the not too distant past of Hungarian paediatricians and school doctors recommended and prescribed American developmental norms dating from the 1940s; since the American data relate to a quite different ethnic group, it is obvious that they could not have been appropriate from a Hungarian aspect.

However, there are few Hungarian data of a similar nature as concerns head measurements, and particularly face measurements (BALI, 1932; DEZSŐ, 1967; EIBEN, 1967; EIBEN and PANTÓ, 1984; RAJKAI, 1967); further, the articles that have been published mainly report the results of measurements of head parameters. This led us to prepare norm tables relating to some head measurements in children, similar to those utilised for the assessment of body development.

## Material and method

This article emerged as a side product of an extensive compilation of data. During the period 1980-84, more than 32,000 girls were examined in a study involving puberty. Since the classes participating in that study were generally coeducational, the boys also took part in the investigation. Overall, in the different parts of the country, head measurements were made on a total 23,338 boys and girls aged 3-18 years. The parameters calculated from the data (mean, standard error and range) were reported earlier (FARKAS and NYILAS, 1988). On the basis of these data, development curves have now been prepared (Figs 1-18).

## Results

The above mentioned development curves provide results that can be used in everyday practice.

We should like to draw attention to some conclusions that can be drawn from the parameters and the curves.

First, as concerns the nomenclature, the expression data collection originates from the incorrect, but frequently applied expression cross-section study. It is more correct to name this data collecting method data collection on one occasion. The essence is that measurements are made on children of various ages and of either sex at a given time. This allows the compilation of a large number of data within a short period. The method has the disadvantage that it does not permit an indication of the developmental trend exhibited by generations born in different years and hence growing up under different economic, ecological and social conditions, which may markedly influence the results. Thus it may arise, for instance, that, in the event of one measurement, the mean for a group of higher age will be less than that for the following age group, which is clearly impossible in repeated measurements on the same children. Accordingly, the development curves constructed from parameters originating from data collected on one occasion must be corrected if they are to reflect the growth trend. However, it is not certain that this correction will result in the true values for a given age group.

Another problem arises in the assessment of the tempo of growth in relation to head measurements. The ratio of head length for neonates is 1:4, whereas for adult it is 1:8. Hence, the head measurements change to a lesser extent than do the body measurements during extrauterine life. A connected point here is that the facial dimensions change more extensively before the emergence of the milk teeth and the permanent teeth. As concerns the facial measurements, it is difficult to delineate such clear cut and well-definable growth stages as in the cases of weight or height, for instance. A graphical plot of the annual increase therefore results in a rather sawtooth curve.

As a third problem, it may be mentioned that the absolute changes in head measurements are much smaller than those in the body dimensions. Accordingly, the difference between the means for two successive age groups is sometimes very slight. This makes practical application of the development values somewhat difficult.



The fourth question is one of viewpoint. In cases of probability calculation and of Gaussian curves, a question of principle arises on the basis of the correlation of the mean and the standard error. For an infinite number of data, it is known that 95% of the cases fall within the interval  $\pm 1,96 \times$  standard error. In biological practice, this is referred to as the normal range. In this way, it is assumed that, for a large sample, 2,5% will automatically be classified as very small (underdeveloped), and 2,5% as very large (overdeveloped). In medical practice, percentiles are preferred in use. Accordingly, 3% of the population is regarded underdeveloped, and 3% as overdeveloped. This means that, at each end of the scale, there are 0,5% of the cases which are still considered to be normal according to the percentiles. This problem primarily arises in connection with growth curves, but it can be eliminated if the normal range is calculated from the formula  $\pm 1,96 \times$  standard error on the basis of the mean and the standard error. In practice it is merely a technical question to decide whether, in a given case, the growth curve or the tabulated values should be taken as basis. Since the construction of the growth curve requires the previously mentioned correction, it is clear that, for an assessment of the actual situation, it is more correct to apply the tabulated data.

We give both forms and leave it to the judgement of the user to decide which to employ.

Our intention in mentioning the above questions was merely to point to some problems that occur in practical life as concerns anthropometric data.

At the same time, we hope that the reported tables and growth curves will be of assistance primarily to odontologists dealing with children as regards the solution of practical problems.

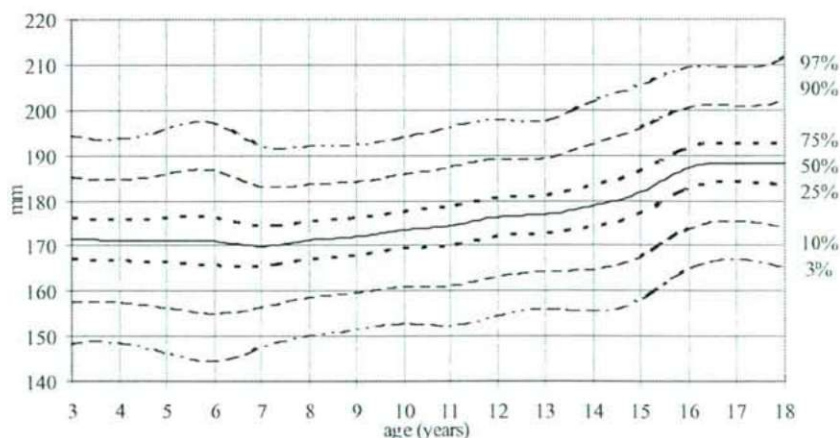


Fig. 1. Percentiles of boys' maximum head length (1)



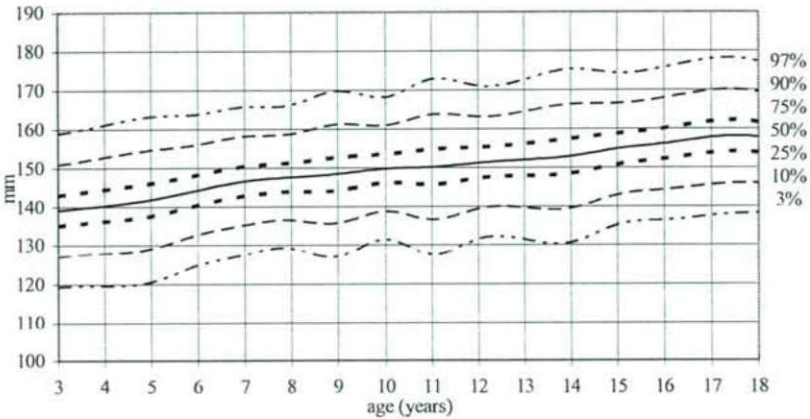


Fig. 2. Percentiles of boys' maximum head breadth (3)

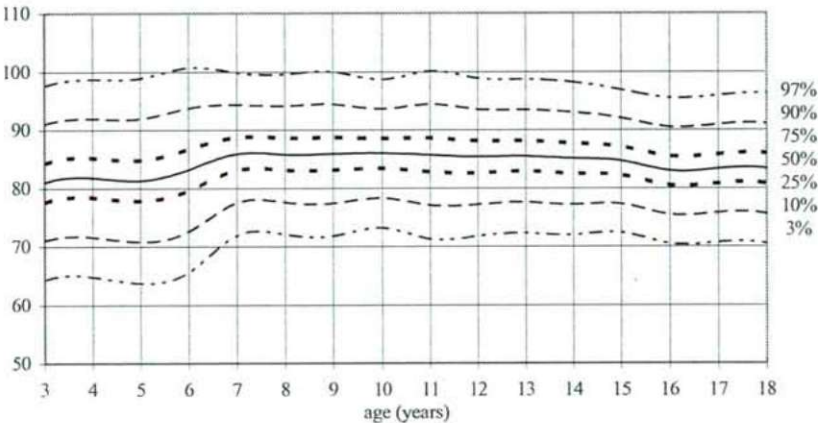


Fig. 3. Percentiles of boys' cephalic index (3:1)

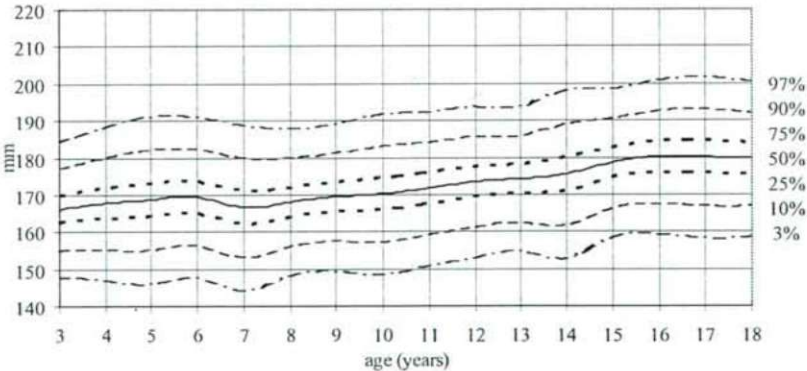


Fig. 4. Percentiles of girls' maximum head length (1)

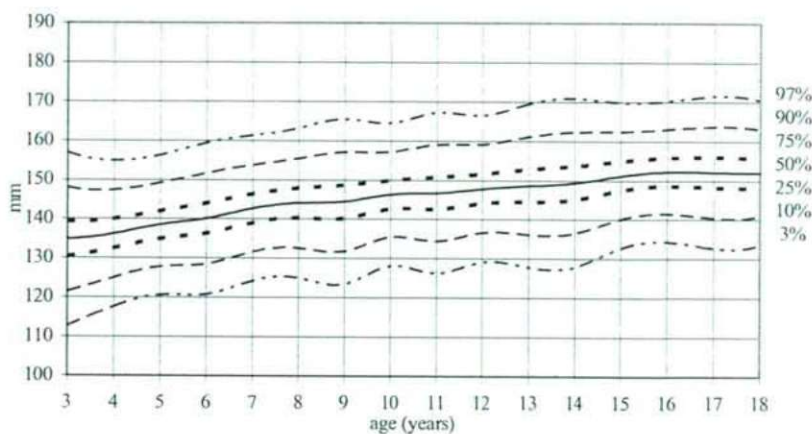


Fig. 5. Percentiles of girls' maximum head breadth (3)

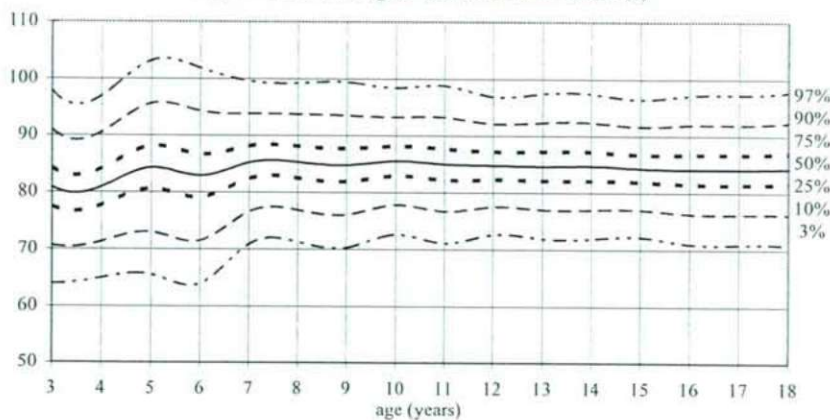


Fig. 6. Percentiles of girls' cephalic index (3:1)

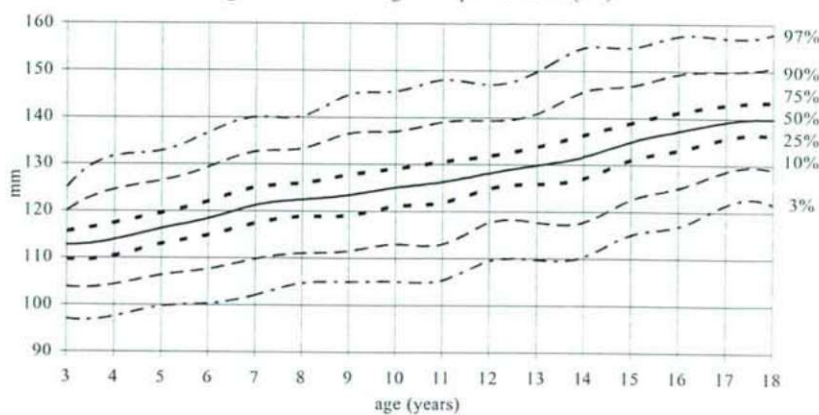


Fig. 7. Percentiles of boys' bizygomatic breadth (6)

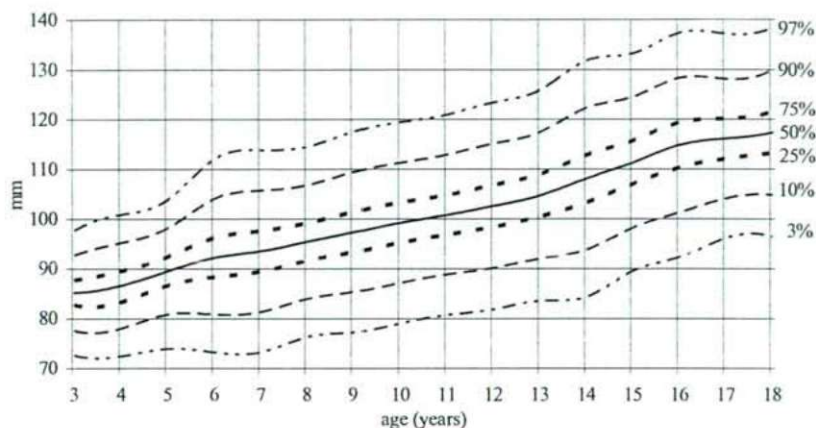


Fig. 8. Percentiles of boys' morphological facial height (18)

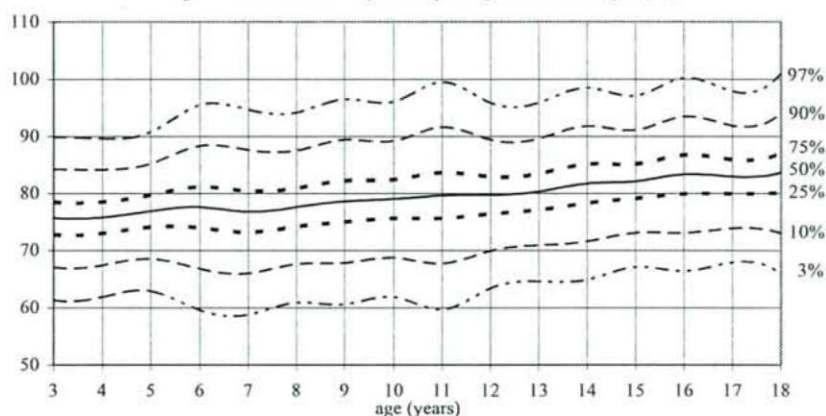


Fig. 9. Percentiles of boys' facial index (18:6)

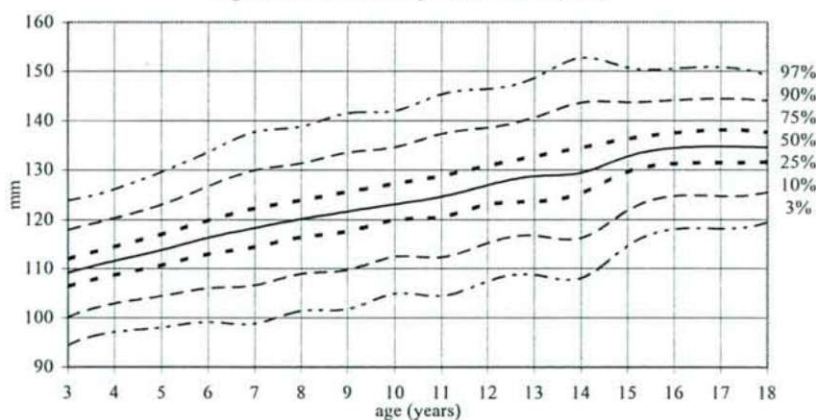


Fig. 10. Percentiles of girls' bizygomatic breadth (6)



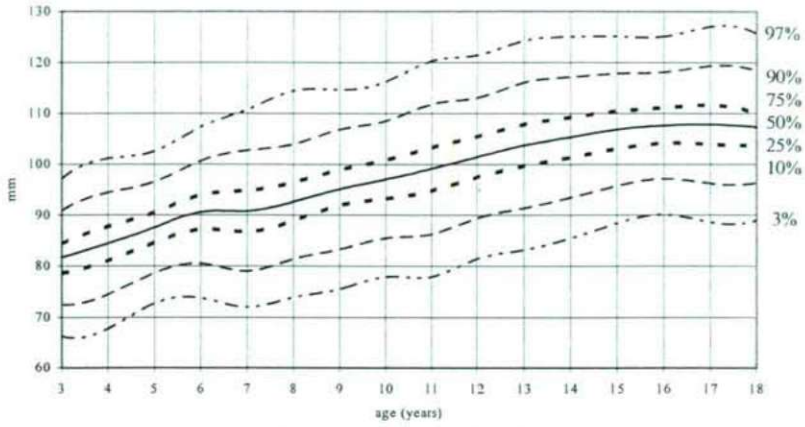


Fig. 11. Percentiles of girls' morphological facial height (18)

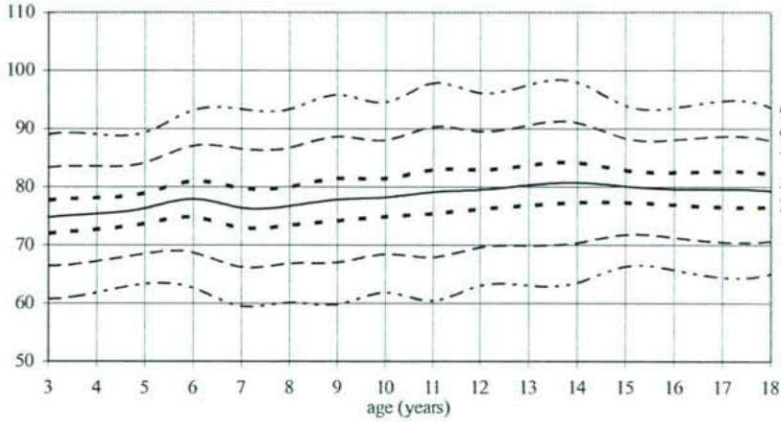


Fig. 12. Percentiles of girls' facial index (18:6)

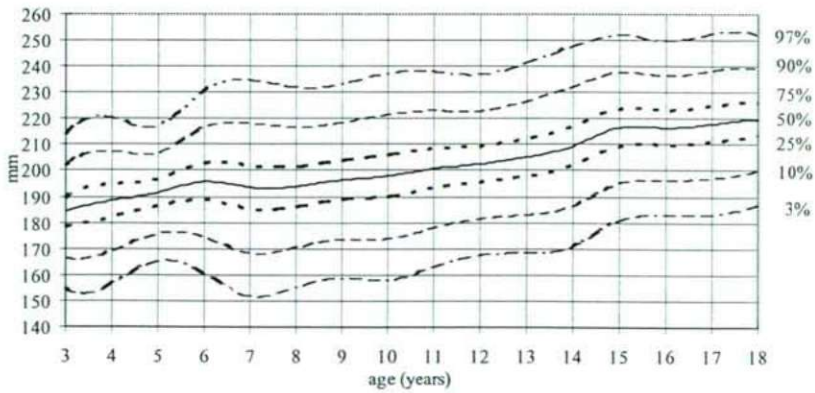


Fig. 13. Percentiles of boys' total head height (16)

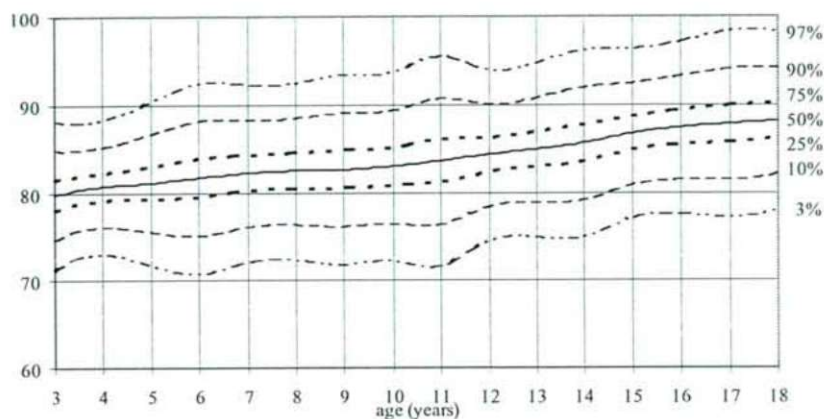


Fig. 14. Percentiles of boys' transverse cephalofacial index (6:3)

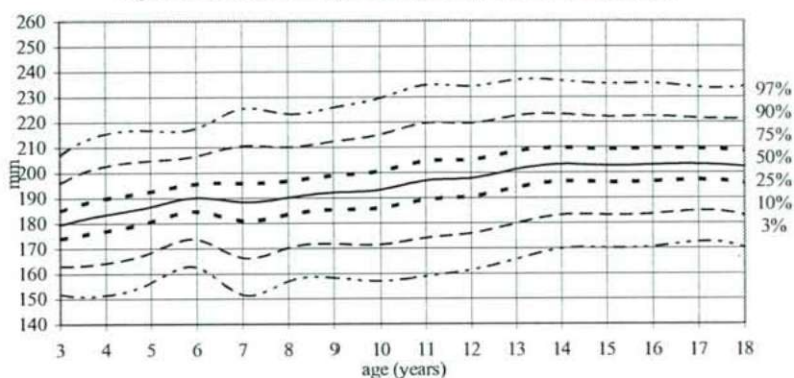


Fig. 15. Percentiles of girls' total head height (16)

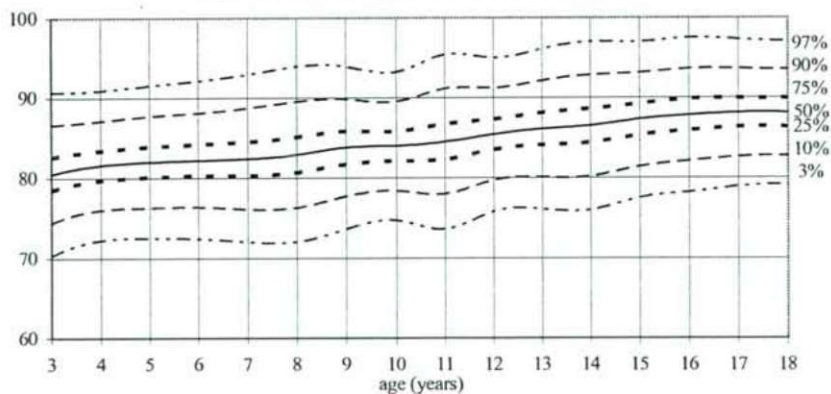


Fig. 16. Percentiles of girls' transverse cephalofacial index (6:3)

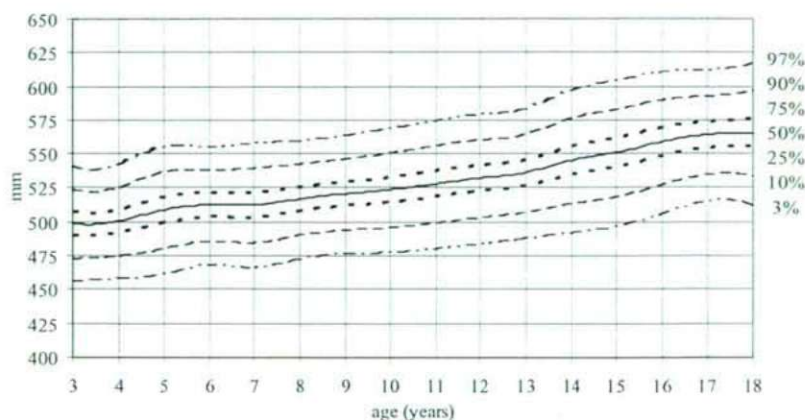


Fig. 17. Percentiles of boys' head circumference (45)

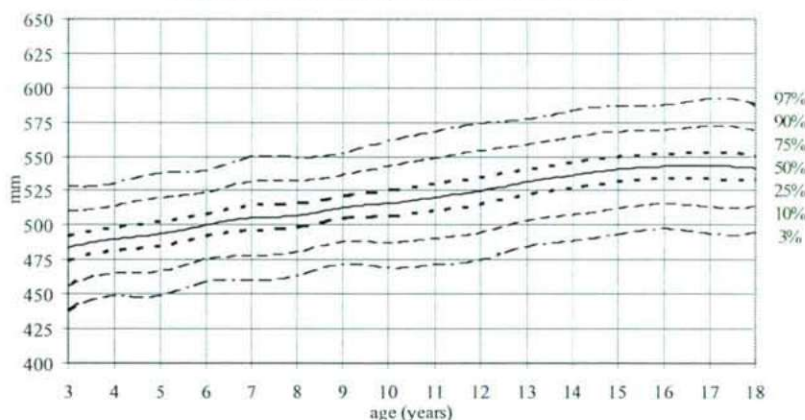


Fig. 18. Percentiles of girls' head circumference (45)

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Short communication

COMPARATIVE INVESTIGATION OF SOME PERIANTH TRIATS  
IN THE TWO MORPHS OF *PRIMULA VERIS* AND *P. VULGARIS*

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(Received: November 20, 1996)

During the last two decades there has been a resurgence of interest in heterostyly. On one hand, studies have been extended to taxa in which heterostyly was previously not investigated, e. g. to species of Boraginaceae (WELLER and ORNDUFF, 1989), Menyanthaceae (OLESEN, 1986) and Turneraceae (BARRETT and SHORE, 1987). On the other hand, recent studies have employed the up-to-date techniques of cytochemistry (HESLOP-HARRISON *et al.*, 1981; DULBERGER, 1987), molecular genetics (CLARKE *et al.*, 1989) and electron microscopy (SCHOU, 1984).

We investigated the morphological features in the distylous plants, as in our preliminary studies we found differences not only in the style length and the stamen position but also in the dimension of perianth characters, such as the lengths of calyx, corolla-tube and corolla-lobes. We examined separated natural populations of *Primula veris* L. and *P. vulgaris* L. in 1996. The aim of this work was to describe the morphological differences of perianth between the two species and between the pin (long-styled) and thrum (short-styled) morphs of the species. We carried out statistical analysis of the data and the means were compared by Student's t-test. We also prepared frequency-distribution diagrams of the characters to reveal the background of the differences.

The comparison of the species shows that the means of corolla-tube length and corolla-lobe length differ significantly in both morphs at a level of significance of 0.1% (Table 1). The most striking difference was found in the corolla-lobe length where the mean value of *Primula veris* was approximately 50% of the mean of *P. vulgaris* in both morphs (Table 2). In the case of the calyx length, a significant difference between the species was found only in the thrum morphs (level of significance is 1%).

The other important difference between the species is that the standard deviation of all the characters examined was higher in *Primula vulgaris* than in *P. veris*, especially in the pin morphs (Table 2).

The comparison of the two morphs shows that it is reasonable to differentiate between the morphs not only on the basis of style length and stamen position. The means of the perianth characters of the morphs also differ from each other in both species. Consequently it is not sufficient to characterize the species by the means

calculated from the whole populations but it is necessary to add the means of each morphs (Table 2).

Table 1. Level of significance of the difference between *P. veris* and *P. vulgaris* and between the pin and thrum morphs of these species (means were compared by Student's t-test).

	<i>P. veris</i> : <i>P. vulgaris</i>			<i>pin</i> : <i>thrum</i>	
	whole pop.	pin	thrum	<i>P. veris</i>	<i>P. vulgaris</i>
calyx length	N.S.	N.S.	1 %	5 %	N.S.
corolla-tube length	0.1 %	0.1 %	0.1 %	0.1 %	0.1 %
corolla-lobe length	0.1 %	0.1 %	0.1 %	1 %	10 %

Table 2. Means and standard deviations of the perianth characters.

	<i>Primula veris</i>			<i>Primula vulgaris</i>		
	whole pop.	pin	thrum	whole pop.	pin	thrum
calyx length	1.78±0.15	1.74±0.14	1.83±0.16	1.73±0.17	1.76±0.18	1.71±0.17
corolla-tube length	1.46±0.15	1.54±0.12	1.38±0.14	1.76±0.17	1.68±0.18	1.84±0.14
corolla-lobe length	0.74±0.11	0.77±0.11	0.70±0.09	1.40±0.15	1.44±0.17	1.37±0.13

The largest difference between the morphs was detectable in the corolla-tube length at a significance level of 0.1% in both species. The calyx length and the corolla-lobe length did not differ significantly between the morphs, or if they did, the level of significance was higher than 0.1% (Table 1).

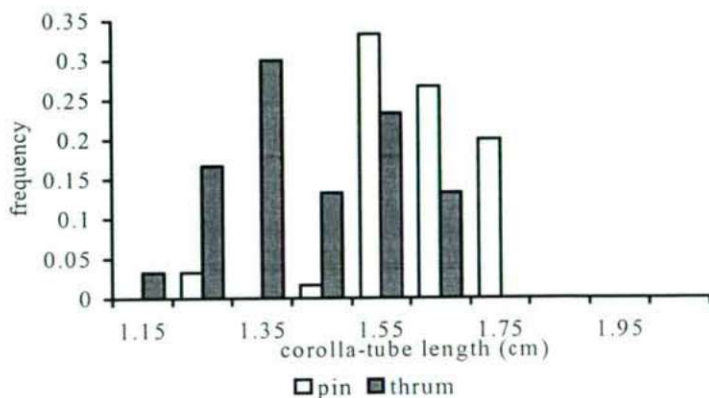


Fig. 1. Frequency-distribution diagram of corolla-tube length in pin and thrum morphs of *Primula veris*

We also prepared the frequency-distribution diagrams of the characters. In the corolla-tube length, the most differing feature, we found that the most frequent category of thrum morphs (category of 1.35 in *Primula veris* and category of 2.05 in *P. vulgaris*) have the frequency of zero in pin morphs (Figs 1 and 2). The diagrams of the corolla-

lobe length are very similar to each other in the morphs while the diagrams of the calyx length are quite random and the difference can not be related unambiguously to the morphs so these features appear not suitable to distinguish the morphs from each other.

On the basis of our results the corolla-tube length and corolla-lobe length seem to be suitable to distinguish the species of *P. veris* and *P. vulgaris* as the means separate from each other quite sharply.

To distinguish the morphs, only the corolla-tube length seems to be applicable on the basis of the means and the difference of the frequency-distribution diagrams.

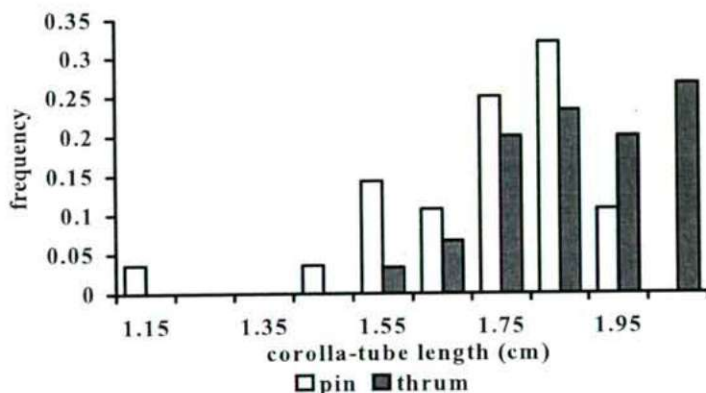


Fig. 2. Frequency-distribution diagram of corolla-tube length in pin and thrum morphs of *Primula vulgaris*

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**Short communication**

**THE OCCURRENCE OF *CRASPEDACUSTA SOWERBYI* LANCESTER  
ALONG THE RIVER TISZA**

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(Received: June 1, 1996)

This fresh-water medusa was first found in the aquarium of the Teachers' Training College in Pécs by BUCHERT in 1956. In the late summer of 1959, both the medusa and the polyp form of the species were found in a backwater of the River Dráva at Őrtilos by EPERJESSY and BUCHERT. The appearance of the species was first reported by WIESINGER in 1959. A detailed description of its development, nutrition and taxonomy according to laboratory examinations was given by BUCHERT (1960).

**Position and habitat of the species**

The effective name of the species was given by DEJDAR in 1934. He ranked it in the Trachylinae suborder and the Olindididae family. The species is wide spread in Europe except the Balkans, and also lives in China and North America (UCHIDA, 1955). BUCHERT found it in various reed-grass vegetation (*Myriophyllum verticillatum*, *M. spicatum*, *Ceratophyllum demersum*, *Utricularia*, *Potamogeton perfoliatus*, *Sagittaria*) and among *Phragmites*. It multiplies by zoogamy and by asexual reproduction. The polyp form displays dimorphism: it has a form with varying numbers of tentacles (8-54) and a form without tentacles. The polyp reproduces the medusa by gemmation. Both the polyp and the medusa live on algae, protozoa, small Crustacea, Rotatoria, Turbellaria and Nematoda. Under laboratory circumstances it also eats *Tubifex* and *Stylaria lacustris*.

**Known occurrence in Hungary**

The medusa form has been detected so far in the backwaters of the Dráva at Őrtilos and Gyékényes, in the gravel-pit lakes at Őrtilos Railway Station, in the Gyékényes-Lankóczi wood in the Bélavár-Palínai wood and in a backwater between Cikolasziget

and Doborgaz in the Szigetköz (as reported by ÁBRAHÁM and SZINETÁR). It has also been found in the lake at Nyékládháza-Debrecen (NÉMETH).

### New habitat of the medusa

The medusa form of *Craspedacusta sowerbyi* was found in the backwater of River Tisza at Tiszadob by ÁDÁM during summer field work on 4th July 1995. It was floating 8-10 metres from the reed bank, next to the reed-grass vegetation in the open water. Some 20 specimens of the species were collected and are stored at the Biological Department of Juhász Gyula Teachers' Training College.

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Short communication

NEW OCCURRENCE OF *ASTROBUNUS LAEVIPES* (CANESTRINI, 1872)  
(ARACHNOIDEA, OPILIONES, PHALANGIIDAE) IN HUNGARY  
(IN THE VALLEY OF RIVER RAKACA — CSEREHÁT)

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(Received: July 15, 1996)

Abstract

A new occurrence of *Astrobunus laevipes* (CANESTRINI, 1872) is found in the North Hungarian Mountain Cserehát, next to river Rakaca, close to villages Szemere and Szászfa. The plant communities including the species are *Cirsio cano-Festucetum pratensis*, *Caricetum elatae*, *Quercetum petraeae-cerris*.

Introduction

The genus first was described by THORELL in 1876 and the species *Hoplites laevipes* by CANESTRINI in 1872.

The species was published by ROEWER (1923) as *Astrobunus meadi*, later by KOLOSVÁRY (1933) as *Roeweriolus hungaricus*, and as *Roeweriolus dudichi* by SZALAY (1951).

SZALAY (1968) published 3 species from the genus (*Astrobunus*) from Hungary in the Opiliones volume of Fauna Hungaria:

*Astrobunus laevipes* CANESTRINI, the species of woodland in Alps; in Hungary along the river Tisza: Tiszadob, Tiszakarád and Szeged.

*A. Meadi* THORELL, the species of wet spring valleys; Transdanube: Kőszeg, Sopron, Sárvár, Csopak, Zirc; Mátra-mountain: Alsópetény; Great Hungarian Plain: Tiszaug, Tiszakarád, Diszadob, Dombrád, Szabolcsveresmart.

*A. Dudichi* SZALAY occurs on the surroundings of Sopron.

### Occurrences - following the time of collection

*Astrobunus laevipes* CANESTRINI: the date and name of the collector is not available, sites: Budapest, Tiszadob, Tiszakarád, Szeged.

*Astrobunus meadi* THORELL: the date and name of the collector is not available, sites: Kőszeg, Sopron, Zirc, Csopak, Tiszaug, Tiszadob, Tiszakarád, Dombrád, Szabolcsveresmart.

*Roeweriolus dudichi* SZALAY: 30. 09. 1943, (the name of the collector is unknown), Tacsai-árok; 27. 05. 1944, 06. 1944, 06. 09. 1944, SZALAY, Sopron- Lőverek; 18. 07. 1944, 06. 1946. SZALAY, Vas-mountain.

*Roeweriolus hungaricus* KOLOSVÁRY: 1925, SZALAY, Sárvár, Csopak; the date and name of the collector is not available, sites: Tiszaug, Tiszadob, Kőszeg, Sopron, Zirc, Csopak.

MARTENS (1968) takes the three species mentioned above a single one as *Astrobunus laevipes*.

It's area: SE Europe, including the countries of the Carpathians, Great Hungarian Plain, the valley between the upper section of river Elba and Danube. In Hungary: in the litter.

### Some specific features of the newly found individuals

Back: light brown basic colour with silver patches on the backplates and the free tergites. 1-1 pair short conical bristle can be found on the tergites I-V and the fifth tergite yet bears 2 smaller bristles in lateral position. On the first free tergite 4 similar conical bristles can be seen from which the size of the two lateral can vary. Two smaller bristles are on the second free tergite.

#### *Description of the habitats*

Cserehát (the part of the North Hungarian Mountain Range) is situated on 300 to 320 m above the sea level. The river Rakaca running from East to West collects the water at the northern base of Cserehát.

The climate of the Rakaca valley is moderately cool and dry, a little bit warmer than the other parts of Cserehát.

Near the villages Szemere and Szászfa (in the Rakaca valley) 113 *Astrobunus laevipes* individuals were collected. The distribution of the individuals varied according to the life cycle of the species. The sampling times are: 05. 1992, 06. 1992, 08. 1992, 09. 1992, 11. 1992 and 08. 1994 (Table 1).

#### *Locality of the sampling*

1. North to the village Szemere. At the beginning of the Kánás-valley on a wet meadow (*Cirsio cano-Festucetum pratensis* community). Along the river toward the stratum spring in a *Filipendulo-Geranium palustre* community, next to it in a

*Caricetum elatae* community and in a zonal turkey oak-sessile oak forest patch (*Quercetum petraeae-cerris* community) growing just at the edge of the river.

2. Near to the village Szászfa — on a wet meadow (*Cirsio cano-Festucetum pratensis* community) close to Német drain canal.

Table 1. Distribution of the individuals among the habitats

Szemere			
1992	sedge vegetation	wet meadow	oak forest
04.22-05.19	1	0	0
05.19-06.19	0	0	0
06.19-08.10	5	2	0
08.10-09.02	3	8	13
09.02-11.03	6	28	15
1994			
04.19-08.23	0	6	18

Szászfa	
1992	wet meadow
04.22-05.19	4
05.19-06.19	0
06.19-08.10	0
08.10-09.02	0
09.02-11.03	4
1994	
	0

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**Summary of thesis submitted for the degree of Candidate of Science**

**THE ROLE OF ETHYLENE IN THE GROWTH AND DEVELOPMENT  
OF BEAN SEEDLINGS TREATED WITH THE GROWTH RETARDANT  
PACLOBUTRAZOL**

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**Introduction**

Plant growth retardants are synthetic growth regulators which reduce the height of plants by inhibiting cell division in the subapical meristems and by reducing the cell elongation in the stem without exerting a substantial effect on the numbers of internodes and leaves.

Plant growth retardants are frequently applied in agricultural technology and horticulture to improve the lodging resistance of cereals, to reduce the height of orchard trees and to save the trimming costs of bushes and hedges. Moreover, growth retardants improve the resistance of plants to abiotic and biotic stresses.

Paclobutrazol ((2RS,3RS)-1-(4-chlorophenyl)-4,4-dimethyl-2-(1,2,4-triazol-1-yl)-pentan-3-ol), a triazole retardant, has been shown to inhibit gibberellin biosynthesis by blocking the oxidation of ent-kaurene to ent-kaurenoic acid. This reaction is catalyzed by cytochrome P-450-dependent monooxygenases, which are inactivated by paclobutrazol.

Plant growth retardants result in a new hormonal balance in treated plants: the concentrations of cytokinins increase as compared to the levels of abscisic acid and ethylene. The ethylene release of tissues decreases in heterotrophic cell cultures, leaf discs or stem segments after paclobutrazol treatment, and it was concluded that ethylene production is inhibited by paclobutrazol as a result of the altered composition of the membrane lipids of retardant-treated cells. These changes in the membrane properties influence the conversion to ethylene of 1-aminocyclopropane-1-carboxylic acid (ACC), the immediate precursor, by decreasing the activity of the membrane-bound ACC oxidase.

We found, however, that bean seedlings treated with paclobutrazol exhibited morphological changes which are evoked or controlled by the plant hormone ethylene, such as

- the increased lateral expansion of the hypocotyl bases of light-grown seedlings,
- the decreased expansion of the leaves,
- modifications in the movement and position of the primary leaves,
- control of adventitious root initiation on cuttings.

The aim of this work was to examine the role of ethylene in the development of these morphological changes in paclobutrazol-treated bean seedlings.

## Materials and Methods

Seeds of bean (*Phaseolus vulgaris* cv. Juliska) were soaked in  $4.25 \times 10^{-7}$  -  $1.7 \times 10^{-5}$  M paclobutrazol (ICI Agrochemicals, Bracknell; UK) and they were grown under controlled conditions in a growth chamber.

The ethylene production of the tissues was determined with a Hewlett-Packard 5890 Series II gas chromatograph equipped with a flame ionization detector and an activated alumina column. The ACC and malonyl-ACC contents of the tissues were measured after chemical conversion to ethylene by the method of LIZADA and YANG. The vacuum extraction of ethylene was carried out by the method of SCHIERLE and SCHWARK.

The indole-3-acetic acid contents of samples were measured spectrofluorimetrically after conversion to indole- $\alpha$ -pyrone, with a Perkin-Elmer spectrofluorimeter. Enzyme activities were determined with a UVIKON 930 spectrophotometer.

## Results

The ethylene production by the organs of paclobutrazol-treated bean plants may be lower or higher than that of the control, or equal to that of the untreated tissues.

In etiolated plants, the ethylene production by the elongation and basal zones of the treated hypocotyls was not lower than in the control, but in the light-grown bean the retardant resulted in a significant reduction in ethylene release by the elongation zone of the hypocotyls. The basal zone of the treated hypocotyls in this latter case exhibited a significant increase in ethylene production as compared to the apical part.

The ethylene production by the blades of the primary leaves was lower in retardant-treated plants in the light, but after a sharp increase at a light/dark transition, this difference was equalized.

The laminar pulvinus and the petiole of the paclobutrazol-treated primary leaves evolved significantly more ethylene than the controls.

During the induction of adventitious roots on the primary leaf cuttings, the ethylene production of the root-forming tissues was not lower in the treated cuttings than in the control, but the kinetics was different.

In those cases where we found an increased ethylene production in the treated tissues, the concentration of ACC, the precursor of ethylene in plant cells, was also higher, which was accompanied by a decreased malonylation.

In the hypocotyls of light-grown seedlings, the retardant treatment induced a lateral swelling in the basal part, without increasing the cell number in the radial direction. This coincided with the increased ethylene production and indole-3-acetic acid content of the tissues. In etiolated plants, paclobutrazol treatment resulted in a shift in the accumulation of ethylene and ACC towards the base of the hypocotyls, identically to the action of white light, which exerted a similar effect in both control and retardant-treated hypocotyls. This means that in the dark paclobutrazol acted similarly to white light. This phenomenon may provide a common basis for the growth-retarding effect of white light and paclobutrazol.

The cell number in the primary leaves was not affected by paclobutrazol. In the leaf blades, the 200% increase in vacuum-extractable ethylene at the light/dark transi-



tion may be regarded as a "natural ethylene treatment" which modifies the expansion of the treated leaves. The effect of ethylene on the leaf expansion may be mediated by peroxidases, which exhibited an increased activity in treated leaves, both in the soluble fraction and in the fractions bound ionically and covalently to the cell wall. Peroxidases secreted into the apoplast catalyze the formation of intermolecular bonds between cell wall polymers leading to the cessation of cell expansion.

The primary leaves of *Phaseolus* display a nyctinastic movement, which is maintained by the cyclic changes in the turgor pressure of the motor cells in the flexor and extensor regions of the pulvinus. The cells of the laminar pulvinus released much more ethylene and contained more ACC in treated plants, which made the night position of the leaf blades permanent by decreasing the auxin activity of the tissues. The indole-3-acetic acid content of the petioles was reduced as compared to the control. The shortage of auxin contributes to the maintenance of the night position of the leaves because the electrogenic pumps of the motor cells are activated by indole-3-acetic acid.

The inhibitors of ethylene biosynthesis or action, aminooxyacetic acid and  $\text{Co}^{2+}$  ion or 2,5-norbornadiene and  $\text{Ag}^+$  ion, respectively, effectively inhibited the development of these morphological phenomena in treated plants, the lateral expansion of the light-grown hypocotyls at the base, and the sleeping position of the primary leaves in the light, and partially reversed the inhibition of primary leaf expansion. These morphological changes can be evoked in control plants by the ethylene generator Ethrel and the precursor ACC which suggests that ethylene is involved in the control of these processes.

Paclobutrazol is frequently used to improve the adventitious root formation on cuttings. In contrast, it was found that the primary leaf cuttings of paclobutrazol-treated bean plants rooted very poorly. The time course of ethylene production during the rooting process revealed that the ethylene production of the root-forming petioles was not lower in the treated cuttings than in the control. The rooting capacity of these cuttings could be restored by applying abscisic acid or Ethrel exogenously on the blades. Treatment with 5  $\mu\text{M}$  abscisic acid resulted in an increased ethylene production and ACC content of the root-forming petioles 48 hours after treatment. The effect of abscisic acid could be reversed by 10  $\mu\text{M}$   $\text{CoCl}_2$  in the rooting solution, which decreased the ethylene production of the tissues by about 50%. It was concluded that the inhibition of adventitious root formation in paclobutrazol-treated primary leaf cuttings could be developed by a relative ethylene deficiency.

On the basis of these results, it was found that the ethylene production by the tissues of paclobutrazol-treated bean plants can be lower than, higher than or equal to that of the control. The ethylene production determines the morphology of the organs, while the effect of ethylene depends not only on the absolute concentration, but also on the physiological and hormonal status of the tissues.

This work afforded new information both on the physiological effects of paclobutrazol and on the role of ethylene in these developmental processes. Data on the control of leaf development and adventitious root formation by paclobutrazol may provide direct information for users of plant growth regulators.



Summary of thesis submitted for the degree of PhD

**THE ROLE OF GABAERGIC INTERNEURONS IN MICROCIRCUITS  
OF THE CAT VISUAL CORTEX**

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**Introduction and aims**

On average, every fifth neuron and every sixth presynaptic terminal in the neocortex synthesizes and presumably releases gamma aminobutyric acid (GABA). Synaptically released GABA is essential for limiting excitability within the cortex to operational levels, to maintain the receptive field properties of neurons in sensory cortex and it plays a critical role in the timing of both subthreshold events and the firing of cortical cells. Postsynaptic effects of GABA in cortex are mediated by fast activating anion channels, the GABA<sub>A</sub> receptors, and by GABA<sub>B</sub> receptors which activate a potassium conductance via a slower G-protein mediated mechanism. There is general agreement that near the soma mainly GABA<sub>A</sub> receptors are activated, whereas GABA iontophoretically applied to the dendrites evokes complex multiphasic responses.

Most of the synaptically released GABA in cortex originates from intrinsic cortical neurons, which differentiated into distinct cell types that appear to target different compartments of the postsynaptic neuron with their terminals. Although very few GABAergic neurons have been characterised quantitatively for their postsynaptic targets, it appears that for example, cortical basket cells innervate the soma and proximal dendrites, and are therefore likely to act through GABA<sub>A</sub> receptors. The postsynaptic effect of neurons that innervate the dendritic domain of neocortical cells is unknown, although it has been suggested, mainly in analogy with results on hippocampal neurons, that synaptically activated GABA<sub>B</sub> receptors are located in the dendritic region. The action of GABA in the dendritic region is of interest because in this domain of the neuron GABAergic mechanisms probably interact with other receptor mediated events and voltage dependent conductances specific to the dendritic region. Recently, microapplication of glutamate was used to evoke GABAergic responses from different sites in the cortex *in vitro* and the results suggested that GABA<sub>A</sub> and GABA<sub>B</sub> receptor mediated responses can be evoked from different sites, which was interpreted as indicating that separate neurons activate GABA<sub>A</sub> and GABA<sub>B</sub> receptors, but the cells were



not identified. Thus, although there is some information about the neuronal classes that release GABA and two major mechanisms of postsynaptic responses have been found in cortical cells, only indirect experimental data link some presynaptic cell types and postsynaptic mechanisms.

The identification of cortical neurons evoking particular postsynaptic effects can be made by recording simultaneously from both the presynaptic and postsynaptic neurons followed by the microscopic visualisation of the pre- and/or postsynaptic cells. Combining this method with subsequent electron microscopic verification of the synaptic junctions mediating synaptic interactions provides an opportunity for the rigorous assessment of differences and similarities in synaptic interactions. In the neocortex, to our knowledge, only one identified and visualised sparsely spiny interneuron has been reported to evoke fast IPSPs in a pyramidal cell, although the sites of interaction between the two cells were not determined.

When addressing experimental questions regarding the functional properties of identified GABAergic neurons, cat visual cortex appears to be suitable for two major reasons. First, detailed work has been done to investigate GABAergic local-circuit neurons whereas in the rat the equivalent cell types remain considerably less well-defined. Second, three decades of intense research have elucidated many of the physiological properties of cat visual cortex. Several of these studies have already highlighted the importance of GABAergic circuits in shaping the functional characteristics of cortical neurons and numerous specific models and wiring diagrams have been put forward, although the majority of them remain to be tested experimentally.

In order to evaluate quantitative differences in the termination patterns of cortical GABAergic cells and to establish the precise placement of synapses that mediate identified postsynaptic effects we used paired intracellular recordings and subsequent light- and electron microscopic analysis of the connections in the first series of our experiments. We examined how the postsynaptic effect of identified GABAergic cells is influenced by the number of synapses between pairs of neurons and the location of synaptic release sites on the target cells.

After the identification of postsynaptic responses evoked by interneurons we examined the activation of cortical GABAergic cells using similar experimental procedures. It is generally accepted that locally activated interneurons are essential to control cortical excitation and circumscribed classes of interneurons may subserve distinct functional roles. However, the GABAergic cell types, targeted by recurrent axon collaterals of pyramidal and spiny stellate cells, are unknown. In the hippocampus, association of GABAergic axons with subsets of excitatory afferents strongly suggests a pathway-specific modulatory function. Not surprisingly, this apparent division of labour is probably paralleled by differences in the excitatory inputs and activation of GABAergic neurons. Due to the geometry of their dendrites certain types of interneurons in the hippocampal molecular layer may only be activated in feedforward manner. Others, such as the somatostatin and mGluR1 $\alpha$ -positive interneurons, receive predominantly, if not exclusively recurrent excitatory input, whereas some cell classes, such as basket neurons, are presumably involved in both types of circuit.

Thus, we addressed the following questions in the second set of our experiments: Which types of interneurons receive local excitatory feedback? Is the recurrent input targeted to a particular domain of the postsynaptic somato-dendritic surface? What are the properties of recurrent unitary EPSPs? What is the strength of individual connections? Which factors contribute to the variability of postsynaptic responses?

Neocortical interneurons receive input from pyramidal and nonpyramidal cells and also from subcortical afferents. In the experiments mentioned above, we labeled synaptically coupled neuron pairs from slices of the cat visual cortex. During the anatomical analysis of such biocytin filled cell pairs, we found, to our surprise, that a significant portion of the light- and electron microscopically detected connections was formed by axons originating undoubtedly from the parent cell. We detected the same result also from preparations containing only one filled neuron.

In 1972, VAN DER LOOS and GLASER proposed the word autapse to describe a synapse between a neuron and its own axon. In addition to their original Golgi study in rabbit neocortex, possible autaptic contacts have been observed in dog and rat cerebral cortex, monkey neostriatum and cat spinal cord with the classical method. Several groups detected such self-innervating connections with intracellular markers from various brain regions, like substantia nigra, striatum and spinal cord. All of these studies were based on light microscopical observations except PETERS and PROSKAUER'S work, which verified an autapse on a multipolar stellate cell and a fraction of recent data by LÜBKE *et al.* on layer V pyramids from developing cortex. Autapses of cell cultures have been used as model for synaptic interactions in several physiological experiments, but a few study proposed *in vivo* functional significance for inhibitory autaptic innervation in the rat neostriatum and in *Aplysia* buccal ganglia.

Using intracellular biocytin labeling and correlated light- and electron microscopy, we determined the exact number of autapses on several neocortical cell types. We found that different cortical cell types exhibit various degree of self-innervation with a subcellular location reflecting the postsynaptic target preference of the parent cells. Moreover, filling synaptically coupled cell pairs it became feasible to compare the number and position of synaptic junctions on a given postsynaptic cell with that of autapses established by the same presynaptic neuron.

## Materials and methods

Brain slices (400  $\mu$ m thick) were obtained from areas 17 and 18 of adult cat visual cortex. The slices were transferred to a recording chamber, where they were maintained at 34–35 °C at the interface between oxygenated artificial cerebrospinal fluid (ACSF) and a humidified atmosphere saturated with 95% O<sub>2</sub> / 5% CO<sub>2</sub>. Recording electrodes were filled with 2% biocytin. Putative GABAergic neurons were identified due to their physiological characteristics, such as short-duration action potentials followed by large amplitude fast afterhyperpolarizing potentials (fAHP). Once a stable recording had been obtained a search was made for cells displaying the electrophysiological properties of pyramidal and spiny stellate neurons. Synaptic coupling was tested using on-line spike-triggered averaging whilst eliciting firing in the interneuron with either depolarizing current pulses or constant DC current injections.

In most of the cases, diffusion, presumably aided by depolarizing (0.1–0.5 nA) current pulses employed during recording resulted in an adequate filling of neurons by biocytin. Slices were fixed and re-



sectioned at 60  $\mu\text{m}$  thickness and the biocytin filled cells were visualised by the avidin-biotinylated horseradish peroxidase (ABC) method with diaminobenzidine as chromogen.

Recovered cells were reconstructed from the serial 60  $\mu\text{m}$  thick sections of the entire slice under a light microscope using a drawing tube. The total number of axonal varicosities in the slice, some of them shown by subsequent electron microscopy to correspond to synaptic boutons, was counted during the drawing procedure. The tissue volume containing the axonal arbour was calculated for each interneuron. The tissue shrinkage factor was determined by stereotaxic injection of wheat germ agglutinin (WGA) followed by the WGA's visualization identical to that of biocytin. In each case, the location of the pre- and postsynaptic cell in the central portion of the slice enabled us to perform nearly full reconstructions of the dendritic arbours. The entire somatodendritic surface of both recorded cells was tested for close appositions with filled axons, each of which were traced back to the parent soma.

Following light microscopic analysis, axon-rich areas were re-embedded for ultrathin sectioning. The sections were scanned in the electron microscope and all biocytin-filled axonal profiles were followed until they formed synaptic contacts. Since all profiles were followed and the plane of the section randomly cuts through the axonal branches, the above procedure ensured a random sample of postsynaptic targets. Each presynaptic terminal that was studied was completely examined in serial sections to establish the number of synapses it formed. Subsequently, all light microscopically detected sites of close appositions between filled axons and labeled somata, dendrites or spines were tested in serial electron microscopic sections. Moreover, all filled somata were serially sectioned completely for electron microscopic analysis to check for the presence of axonal branches which may have been obscured by the opaque cell bodies.

We applied *a priori* and *a posteriori* cluster analysis using the postsynaptic elements as variables to determine the interneuron cell classes. FISHER'S exact test for heterogeneity was used to compare the frequency of postsynaptic elements amongst the targets of different presynaptic cells. The non-parametric MANN-WHITNEY U-test was applied to compare the properties of the different cell types.

## Results and conclusions

### *Fast IPSPs elicited via multiple synaptic release sites by different types of GABAergic neuron in the cat visual cortex*

All smooth dendritic cells established type II synapses previously shown to be made by terminals containing GABA, therefore the studied cells are very likely GABAergic. Three classes of presynaptic cell could be defined, based on their efferent synaptic target preference determined from random samples of unlabeled postsynaptic cells. *a) Basket cells* ( $n = 6$ ) innervated mainly somata ( $49.9 \pm 13.8\%$ ) and dendritic shafts ( $45.2 \pm 10.7\%$ ) and, to a lesser extent, dendritic spines ( $4.9 \pm 4.6\%$ ). *b) Dendrite-targeting cells* ( $n = 5$ ) established synapses predominantly on dendritic shafts ( $84.3 \pm 9.4\%$ ) and less frequently on dendritic spines ( $11.2 \pm 6.7\%$ ) or somata ( $4.5 \pm 4.7\%$ ). *c) Double bouquet cells* ( $n = 4$ ) preferred dendritic spines ( $69.2 \pm 4.2\%$ ) to dendritic shafts ( $30.8 \pm 4.2\%$ ) as postsynaptic elements and avoided somata.

Interneurons formed  $5240 \pm 1600$  (range 2830-9690) synaptic junctions in the slices. Based on the density of synapses made by single interneurons and the volume density of GABAergic synapses, it was calculated that an average interneuron provides  $0.45 \pm 0.13\%$  of GABAergic synapses in its axonal field.

The location of synaptic junctions on individual identified postsynaptic cells reflected the overall postsynaptic target distribution of the same GABAergic neuron. The number of synaptic junctions between pairs of neurons could not be predicted from light microscopic examination, the number of electron microscopically verified synaptic sites were generally smaller for the dendritic domain and larger for the somatic domain,



than expected from light microscopy. All presynaptic cells established multiple synaptic junctions on their postsynaptic target cells. A basket cell innervated a pyramidal cell via 15 release sites, the number of synapses formed by 3 dendrite-targeting cells on pyramidal cells were 17 and 8 respectively, and 3 on a spiny stellate cell; the interaction between a double bouquet cell and a postsynaptic pyramidal cell was mediated by 10 synaptic junctions.

All three types of interneuron ( $n = 6$ ; 2 for each type of cell) elicited short-latency IPSPs with fast rise time (10–90 %;  $2.59 \pm 1.02$  ms) and short duration (at half-amplitude  $15.82 \pm 5.24$  ms), similar to those mediated by GABA<sub>A</sub> receptors.

Average amplitudes of unitary IPSPs ( $n = 6$ ) were  $845 \pm 796$  mV ranging from 134–2265 mV. Variability of IPSP amplitude was moderate, the average ratio of IPSP and baseline noise variance was  $1.54 \pm 0.96$ . High frequency activation of single presynaptic dendrite targeting cells led to an initial summation followed by use-dependent depression of the averaged postsynaptic response. Double bouquet cell evoked IPSPs, recorded in the soma, had a smaller amplitude than those evoked by the other two cell types. In all connections, transmission failures were rare or absent, particularly when mediated by a high number of release sites.

The results demonstrate that different types of neocortical GABAergic neurons innervate distinct domains on the surface of their postsynaptic target cells. Nevertheless, all three types of cell studied here elicit fast IPSPs and provide GABAergic input through multiple synaptic release sites with few, if any failures of transmission.

*Effect, number and location of synapses made by single pyramidal cells onto aspiny interneurons of cat visual cortex*

Pyramidal neurons in layers II/III elicited monosynaptic EPSPs in three distinct categories of smooth dendritic local-circuit neurons, namely basket cells ( $n = 3$ ), a dendrite-targeting cell ( $n = 1$ ) and a double bouquet cell ( $n = 1$ ). Unitary EPSPs in basket cells were mediated by 1, 2, and 2 synaptic junctions, whereas the pyramid-to-dendrite-targeting cell and pyramid-to-double bouquet cell interaction were mediated by 5 and 7 synaptic junctions, respectively. Synaptic junctions were found on all somato-dendritic compartments, with a tendency to be clustered in individual connections. Two pairs were reciprocally connected.

Unitary EPSPs had an average amplitude of  $1,005 \pm 518$  mV, fast rise times (10–90 %;  $0.67 \pm 0.25$  ms) and were of short duration (at half-amplitude  $4.7 \pm 1.0$  ms). Their decay was monoexponential ( $\tau = 7.8 \pm 4.3$  ms) and, at hyperpolarised membrane potentials, appeared to be shaped by passive membrane properties ( $\tau = 9.2 \pm 8.5$  ms). All parameters of concomitantly recorded spontaneous EPSPs (mean rise time =  $0.68 \pm 0.18$  ms; mean duration =  $4.7 \pm 1.7$  ms; mean amplitude =  $981 \pm 433$  mV) were remarkably similar.

By the analysis performed by OLE PAULSEN and CHRISTIAN STRICKER, the amplitude fluctuations of the EPSPs could be accounted for by a quantal model of transmitter release. Without quantal variance, however, the minimum number of components in the model, excluding the failures, exceeded the number of electron microscopically determined synaptic junctions for all five connections. In contrast,

incorporating quantal variance gave a minimum number of components which was compatible with the number of synaptic junctions, and which fitted the data equally well as models incorporating additional components but no quantal variance.

In conclusion, at least three distinct interneuron classes receive local excitatory pyramidal cell input which they relay to different compartments on their postsynaptic target neurons. Unitary EPSPs show fluctuations that can be accounted for by a quantal model of transmitter release, when incorporating quantal variance. The reliability of transmission is high, while the fast time-course of the EPSPs constrains their temporal summation and predisposes these classes of interneurons to act as coincidence detectors.

*Massive autaptic self-innervation established by neocortical interneurons*

Self-innervation was light microscopically tested on 10 pyramidal, 7 spiny stellate cells and on 39 smooth dendritic interneurons from cortical layers II-V. Putative autapses ( $n = 171$ ) could be observed on each smooth interneuron and on 7 pyramidal cells, but not on spiny stellate cells. After the electron microscopic evaluation of all putative sites, 134 autapses could be verified, but the correctness of light microscopic estimation varied between cell types.

Pyramidal cells showed rare (10 %) and relatively weak self-innervation as only one pyramidal cell innervated itself with two autapses on the same dendritic spine, in spite of the examination of ultrathin sections from all ( $n = 25$ ) predicted sites on the pyramids.

After the classification by the postsynaptic target distribution, all putative autapses of 6 basket ( $n = 68$ ), 3 dendrite targeting ( $n = 58$ ) and 2 double bouquet cells ( $n = 20$ ) were scrutinised. Self-innervating junctions could be verified on each basket ( $n = 66$ ;  $11 \pm 7$  per cell) and dendrite targeting ( $n = 66$ ;  $22 \pm 12$  per cell) cells, but none of the double bouquet cells formed autapses. Although autapses were identified on all parts of the somatodendritic domain on both cell classes, basket cell autapses were significantly closer ( $13.3 \pm 23.2$  mm) to the soma, than autapses established by the dendrite targeting cells ( $51.8 \pm 49.9$  mm).

In summary, neocortical cell types establish various degree of self-innervation. Unlike on spiny cells, autapses are abundant on basket and dendrite targeting interneurons with similar subcellular target preference to that of synapses of the parent cell. The extensive self-innervation may modulate local dendritic information processing and intrinsic excitability via inhibitory feedback.



**Summary of thesis submitted for the degree of Candidate of Science**

**SPATIO-TEMPORAL PATTERNS AND PATTERN TRANSFORMATIONS IN  
SAND GRASSLAND COMMUNITIES**

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**Introduction**

Investigation of the vegetation of an area used to be started with a general description and identification of vegetation units, i.e. definition of the cenological state of vegetation stands. Cenological analysis of plant communities is based on the idea that the associations are natural units of the vegetation. They have been believed more or less homogeneous and static, discrete and distinguishable.

The series of heavy controversy, that started at the beginning of this century and has not been finished up till recently, pointed out that the plant communities can not be considered as discrete units as the individuals of a species. The spatio-temporal relations among community units are of quite different nature because they have no definite envelope, "skin", or this envelope is rather diffuse, and on the other hand they represent a much more complicated level of organization.

Distinction of plant communities in the field sometimes seems easy when the differences — physiognomic, textural — between two stands are significant, abrupt changes can be detected in their border zone. These ecotones form a real border between adjacent stands, but the units overlap for a certain degree. In the ecotones of very different communities — e.g. forest-meadow, aquatic vegetation-shoreline vegetation etc. — there can be discovered the scaling problem that the border line at a larger scale which is appropriate for vegetation mapping, will become more and more diffuse when smaller and smaller scales are used. At a scale of population or coalition, the detection of the border is quite dubious.

In the case of communities with very similar architecture, ecotones can not be recognized, and the objective spatial distinction between communities is very difficult. This fact led to the development of continuum concept of the vegetation. But indisputable that the cenological structure and the ecological state of two communities without definite border can be surely distinguished. However the question about the spatial pattern transformation in the border zone remains open.

Psammophyle grassland communities proved to be good objects for many ecological investigations. Their cenological structure is not too difficult and is rather well known. Their successional pathways are reticulate so they support various pattern transformations. The species number is low in these communities, patch size of the



mosaic like stands is small, so the cost of sampling process is low. The most important phenomenon of these communities in relation to the above problems is that their architecture is very similar.

The goal of this study was to contribute to the knowledge of scaling problems of patterns and processes. The following questions were set up:

What properties should be the basis of distinction of plant communities?

How do grassland communities scan and respond to an inhomogeneous habitat?

What is the spatial pattern of the communities, and what about the transformation of this pattern?

What are the properties of the transitional zones, and what is their temporal dynamics?

## Material and methods

### *The study site*

Investigations were carried out at the research field of the Ecological Department of JATE University, in the Bócsa-Bugac region of Kiskunság National Park. The sample area was a 2.4 ha isolated part of a pasture that was fenced in 1976.

The soil of this grassland is loose sand and humic sand. A mosaic like pattern of sand grassland associations has been developed at the varying relief of the research area, and the size of the stands is usually small, the scale of vegetation units is 10 m. The largest difference of the elevation at the area is about 3 m, and the most frequent elevation difference is 1 to 1.5 m. This habitat enables in this way the frequent occurrence of transitional regions.

The xerothermic associations of the upper relief show a large architectural similarity by the vegetation cover (percentage cover values are 40 to 60 %) and by the average height (25 to 30 cm) of the vegetation. The studied communities should be distinguished syntaxonomically, but their physical border is not marked, not as conspicuous as the transition of even the mesophytic and xerothermic stands.

The following plant communities were identified at the sample area:

*Festucetum vaginatae danubiale*  
*Potentillo-Festucetum pseudovinae*  
*Brometum tectorum*  
*Molinio-Salicetum rosmarinifoliae*  
*Lolio-Potentilletum anserinae*  
*Cynodonti-Poëtum angustifoliae*  
*Achilleo-Festucetum pseudovinae*

The first four associations grew at the fenced area, while the others occurred only at the lower relief of the grazed field. Stands of *Brometum tectorum* occurred mainly on disturbed region, and their proportion was rather low, that is why this community was neglected.

### *Sampling methods*

Most samples were taken from a 2.4 ha isolated part of a grazing field, but some of them originated from the nearby area of the pasture. Three important associations were studied in detail that are separated by the elevation, and show a distribution correlating with disturbance pattern. Studied associations are: *Festucetum vaginatae danubiale*, *Potentillo-Festucetum pseudovinae* and *Molinio-Salicetum rosmarinifoliae*. This sequence means also the elevation distribution, from the upper relief to the lower one. *Potentillo-Festucetum pseudovinae* developed at regions with stronger disturbance — intensive grazing — but the other two communities prefer undisturbed areas.

Relevés were taken for determining the structure and cenological state of the associations. Sampling unit size was 2 × 2 m, and the percentage coverage of each species was recorded. Sampling periods were usually in spring, early summer and autumn, depending on the phenological state of the vegetation.

Temporal dynamics of the vegetation was surveyed in permanent quadrates of 14 places. Number of sampling places in an association was proportional to the proportion of the area of the association, therefore 3 places were established in *Festucetum vaginatae*, 7 in *Potentillo-Festucetum pseudovinae* and 3 in *Molinio-Salicetum rosmarinifoliae*. Only 1 sampling place belonged to *Brometum tectorum*, but it was not included in the evaluation of the results.

Behaviour of border zones of the communities was studied with belt transect method. I used a 40 m long transect, 1 m wide, consisting of 1 m<sup>2</sup> contiguous cells. Percentage coverage of species was recorded from each seasonally, during three years. This transect was laid along an orographical gradient because I assumed the best detection of transitions in this way.

### Statistical methods

Relevés were analysed with multivariate methods. I made the numerical classification with agglomerative cluster analysis, where resemblance functions were percentage similarity and Euclidean distance, respectively, and clustering algorithm was average link (UPGMA).

Multivariate analysis was completed with principal component analysis (PCA) on the basis of correlation matrix of raw data. In the PCA scatterplot, the objects classified in the same community or belonging to the same sampling period were encircled, and in the case of time series trajectories of the centres of sample groups were shown, respectively. Coordinates of group centres were given by the averages of object scores belonging to certain PCA axes. In the scatterplot of transect samples, points representing the adjacent sampling units were connected.

I made only ordination analysis in the case of time series, and the objects or centres of groups were connected according to their chronosequence.

Diversity values were calculated with SHANNON-WEAVER index, and diversity ordering was performed on the basis of RÉNYI index. Diversity values were plotted against  $\alpha$  parameter.

I used the following computer softwares: SYN-TAX III, NuCoSA and Div. Ord.

Climate diagram was drawn from data of Meteorological Station Kecskenét.

### Summary of new result

In sand grassland habitats, on the basis of relief differentiation of stands of the three main plant communities only the *Molinio-Salicetum rosmarinifoliae* association could be distinguished. Elevation position of stands of *Festucetum vaginatae* and *Potentillo-Festucetum pseudovinae* is overlapping, their separation is difficult, but they can be distinguished with cluster analysis.

Separation of the stands on upper relief was affected by microtopographic conditions. While *Potentillo-Festucetum pseudovinae* stands were situated on larger flat areas — sometimes with light slope —, *Festucetum vaginatae* could be found on sand hills of steeper slope.

Maximum cenological distance of the studied communities decreased during the long term study. At the same time, the species number of each stand increased, relative increase was larger at the second half of that period. This could be explained with the homogenizing effect of the extreme environmental conditions.

Each of the seven stands of *Potentillo-Festucetum pseudovinae* community showed similar behaviour in the PCA-space. This was because the changes in species composition were similar. The subgroups did not overlap in the three dimensional factorial space, and the alteration of vegetation structure meant a successional process. The most "linear" structure transformation could be detected in the case of *Molinio-*



*Salicetum rosmarinifoliae*, while the "movement" of *Festucetum vaginatae* stands is rather small. *Potentillo-Festucetum pseudovinae* association was in an intermediate ecostate.

Dendrograms clearly show the significant separation of the first year samples, and the sharp changes related to those. Changes of the xerothermic grasslands between the second two years were slight, but more marked in the case of *Molinio-Salicetum rosmarinifoliae*. I observed a delayed response of later community, because its stands were in more favorite microclimatic conditions.

Species-cover diversity gradually increased in each of 14 stands, and this reflects — together with the increase of species number — to the changes of environmental factors, that resulted in the decrease of community stability.

Changes of climatic factors resulted in the most pronounced structural alterations in the stands of *Molinio-Salicetum rosmarinifoliae*, since those had the highest water demand. Because of the very precipitation poor period, their species composition tended to that of *Potentillo-Festucetum pseudovinae*. Stability of the community of wind grooves was the weakest among the given environmental changes.

Successional changes of stands of *Festucetum vaginatae* were more moderate than those of the other two communities. This could be because *Festucetum vaginatae* stands have grown under extreme circumstances, and they could have the highest tolerance to drought period, so their stability was the strongest among the given circumstances.

Isolation played a very important role in the successional changes of the fenced area of sand pasture community, *Potentillo-Festucetum pseudovinae*, that resulted in the significant decrease of vegetation cover, but the climatic changes determined the trends of secondary succession of this community.

Average of W indicator values showed a strong temporal change in *Molinio-Salicetum rosmarinifoliae*, i.e. the largest decrease of average water demand could be measured in this community. W-values of disappearing species were larger than those of new species. Average W-value of species leaving the community until 1987 was 4.33, while that of newcomers was only 2.13. This trend could be detected also in the case of *Potentillo-Festucetum pseudovinae*, but the differences were smaller. The new species appearing in the arid period were mostly annuals, or perennials with lower water demand.

Trajectories of changes in the PCA factorial space were different for the studied communities. Movements — distance and direction — of the points representing the stands of *Potentillo-Festucetum pseudovinae* were similar. Principal component scores correlated with some structural and environmental features, the closest relationship was computed between W-values and scores on the 1st axis.

The points of *Festucetum vaginatae* showed only a small fluctuation along the 1st PCA-axis, because its structure was hardly influenced by drought.



The three stands of *Molinio-Salicetum rosmarinifoliae* performed a rather different movement in the factorial space. Starting points were close to each other, but later they moved off significantly. Only in the case of two stands could be shown closer correlation between component scores and average W-values. The third point, however, only fluctuated in a small extent, because it was situated at the lowest relief of the sample area, where the water regime of the soil could remain favourable for longer time, that is why this stand could tolerate the precipitation deficiency.

Only weak correlation was between the W-averages and annual precipitation in each community. These refer to the alteration of the water regime of the area, but do not prove clearly direct effect of precipitation distribution on structural changes.

The species joined in three separate groups on the basis of interspecific correlations. The pairwise correlations were positive in two groups, but in the third one there was either no correlation or negative correlation between species. According to these groupings, only two units of the vegetation could be recognized.

Dominance distribution of the species is the basis for visual assessment of boundary zones. Vegetation of the studied transect was species-poor, only 6 to 8 species were dominant, and about the half of the species was distributed on a wider range of the transect. Occurrence of several species was sporadic or in smaller patches. Distribution of many species was overlapping or they showed a continuous transition among vegetation patches. These species had positive interspecific correlation, and they are not suitable to locate the community border of xerothermic grasslands.

Neither the species turnover marked the border zone between xerothermic communities because there were no abrupt changes in species composition, and these two associations had several common species and similar environmental conditions. It was more pronounced in the wind groove region.

We can conclude from transect data, that most species had their own characteristic distribution along the transect. Populations either formed continuous stands or had smaller gaps because of the topography. Distribution pattern of species were developed by their vegetative and generative spreading, that were modified by the environmental factors — mainly soil moisture — and interspecific interactions.

Distribution ranges of the species belonging to the same community were very different, and their overlaps supported a continuous transition among associations. Even the dominant species of neighbouring stands overlapped forming transitional zones of different width. This zone was 2 to 3 m wide between *Potentillo-Festucetum pseudovinae* and *Molinio-Salicetum rosmarinifoliae*, but often much wider between the two xerothermic communities.

To determine the boundary zone between two associations, we must use several methods, and their joined results should present the best approximation of position of borderline (-zone). These methods should be (1) visual assessment on the basis of vegetation structure; (2) assessment from distribution pattern of dominant species; (3) assessment from spatial species turnover; (4) assessment from multivariate analysis of species composition and environmental factors. In general the first two methods do not

give a sharp boundary, but the others can, and the median of the resulted border zone should be considered as the border line between two communities.

Reasons of above processes should be searched in certain populations, and mainly in perennials. Dominance structure of species changes during the vegetation period. Cover values of species depended on their life cycle and on environmental conditions. Two main behaviour types of perennials could be distinguished. *Festuca pseudovina* represented one type, both its cover values and its presence in the quadrates fluctuated, and as a result, its distribution range along the transect was very changing on both sides.

Another type was represented by *Potentilla arenaria*. Its presence in the transect cells was more or less constant, though the cover values changed a little bit. The important property of this type is the gradual disappearance from the border zone of dry communities. In respect of response to the background factors, I called the first type "fast species", and the later one "slow species". Fluctuation and resilience of communities is probably due to "fast" species, and the successional processes should be caused by "slow" ones even if the later are subdominant. Such alterations of spatial distributions of populations cause the seasonal fluctuation of community boundaries.

**Thesen zur Dissertation einer Kandidaturgraduierung**

**ZUR SYNÖKOLOGIE REGIONALER SCHNECKENGEMEINSCHAFTEN,  
TIERGEOGRAPHISCHE UNTERSUCHUNGEN IN DER UNGARISCHEN  
TIEFEBENE UND IM BÜKKGEBIRGE**

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**Wissenschaftliche Aufgabenstellung**

Diese Arbeit faßt 35-jährige ökologische Untersuchungen im Gebiet der Theiß-nahen Ungarischen Tiefebene und im Bükkgebirge zusammen. Die durch frühere, zumeist sporadisch und mit faunistischer Zielstellung durchgeführten synökologischen und ökologischen Untersuchungen führten zu Befunden über die Schneckenfauna und lassen nach ihrer Aufgliederung Einflüsse durch abiotische Faktoren erkennen, betonen insbesondere die Bedeutung der Flüsse bei der Arten-zusammensetzung der Vegetation, weisen auf eine Parallelität zwischen artspezifischer und struktureller Veränderung dieser Regulative hin. Hauptaufgabe ist die Kenntnis der regionalen Zusammensetzung der Schneckenfauna im Verhältnis zur Umwelt sowie eine Bewertung der natürlichen und kulturell bedingten Veränderungen.

**Methoden und wissenschaftliche Zielstellung**

*Geschichte der Erforschung der Schneckengemeinschaften*

In der Malakologie ist die Sammlung von Material Werkzeug und Methode zugleich, schließt somit Zoologie, Ökologie und Tiergeographie mit ein und es besteht ein Zusammenhang zwischen allgemeiner Entwicklung und Zielstellung. Es bestand die Notwendigkeit zur Herausbildung richtiger Methoden auch in der Malakologie. Demnach kommen eine qualitativ relative (Bodenfalle, Zeitsammlung) sowie eine quantitativ absolute (Quadraterfassung) Sammelweise als einmalige Form diesen Bedürfnissen nicht entgegen, da sie einen Vergleich der unterschiedlichen mittleren Kombinationen hinsichtlich der realen lokalen Abundanz nicht gestattet. Am brauchbarsten scheint für eine vergleichende Massencharakterisierung die 10-fache Quadraterfassung zu sein. Diese absolute Methode wurde bereits bei Untersuchungen des Artenbestandes im Pleistozän und Holozän verwendet und erweckte das Interesse in der tiergeographischen Forschung.



Eine statistische Klassifikation und systematische Auswertung der Sammlungen erfolgte in Amerika in den 70-er Jahren und in Europa in den 80-er bis 90-er Jahren. Der Verfasser verwendet für seine Analysen eine seit 1977 verwendete Klassifikation. Die in der heimischen Malakologie und Ökologie erfolgten Fortschritte in der Materialsammlung und die Grundlagen der Datenerfassung wurden bereits früher veröffentlicht.

### *Geschichte der Erforschung in der Ungarischen Tiefebene und im Bükkgebirge*

Hier erfolgt eine Zusammenstellung der vollständigen Artenliste dieser beiden Landschaftsgebiete sowie deren Analyse. In beiden Landschaften begann die Erforschung der Landschnecken im Jahre 1868. Die Arbeiten in der Ungarischen Tiefebene erfolgten auch außerhalb der gegenwärtigen Grenzen. Die Erforschung beschränkte sich in der Anfangsperiode auf das alleinige Sammeln. Sammlungen aus und an der Theiß machte HORVÁTH. Aus tierhistorischer Sicht faßte SOÓS die Ergebnisse zusammen. Nach ökologischen Gesichtspunkten sind die Arbeiten von ROTARIDES und HORVÁTH bedeutend. ROTARIDES führte die Rauminhaltssammlung, VÁGVÖLGYI und AGÓCSI die Quadratmethode ein.

Mit der 1956 systematisch beginnenden Theiß-Forschung begann dann auch die ökologische Erforschung. In den 60-er Jahren führten Schüler von HORVÁTH diese Landschaftsuntersuchungen durch und PINTER begann mit systematischen Arbeiten. Die in diesem Gebiet gesicherte Artenzahl erhöhte sich von anfänglich 42 auf 104 Arten in den 90-er Jahren. Hierzu wurden 120 Arbeiten veröffentlicht.

Im Bükkgebirge waren bis in die 70-er Jahre mehr als 53 Sammler an der Erstellung sporadischer Sammlungen beteiligt. Das Material wurde in den Jahren 1976-77 von VARGA zusammengestellt. Unter Beteiligung von HORVÁTH begannen seit 1950 systematische hydrobiologische Untersuchungen. Bis 1977 wurden so in 58 Arbeiten 71 Arten aus diesem Gebiet vorgestellt. Systematische ökologische Arbeiten sind zwei gesonderten Programmen zu verdanken: dem seit 1973 laufenden MAB Projekt (Sikfökt) und dem seit 1980 in Kraft getretenen Programm: Mensch und Biosphäre, in dessen Rahmen bis zum Jahre 1990 komplexe malakologische Arbeiten erfolgten. Die Ergebnisse wurden in eignen Veröffentlichungen vorgestellt.

### *Untersuchungsmaterial, Methoden*

Die Geländeuntersuchungen erfolgten seit 1958 unter Verwendung eines Quadrats mit den Maßen von  $10 \times 25 \times 25$  cm in bestimmten Vegetationseinheiten. Die untersuchten Waldgebiete bestanden in der Mehrzahl aus intensiv genutzten Wäldern, daneben aber auch aus zwei- bis dreijährigen Pflanzungen. Zusätzlich zur Feldarbeit wurden Daten zum Biotop und zur Bodenzusammensetzung (Ergebnisse von Bodenproben aus Fachlaboratorien) einbezogen. In der Ungarischen Tiefebene wurden 17 Pflanzengesellschaften in 273 Wäldern und Graslandschaften, im Bükkgebirge 36 Pflanzengesellschaften in 175 Wäldern, Graslandschaften und Strauchregionen untersucht. Zusätzlich erfolgten mehrjährige Untersuchungen über Fluktuationen und saisonale Änderungen in Auwald- und Sumpfwald. Mehrjähriges Material stammt aus Boden-

fallen aus dem Gebiet um Sikfökt (13 Jahre), aus Rejte und dankenswerterweise durch LOKSA aus Bockerek. Aus dem Gebiet der Donau, der Theiß sowie deren Nebenflüssen wurden 24 Sedimentssammlungen analysiert. Die Gesamtzahl untersuchter Individuen beträgt 86000.

Alle analytischen und klassifikatorischen Bestimmungen wurden an diesem Gesamtmaterial durchgeführt. Für assoziative Analysen wurden die Bodenvegetation und für Schnecken die mittlere Streuungsfläche mit Temperatur- und Feuchtigkeitsdaten verglichen. Die Determination der Pflanzengesellschaften, die Analyse der Sukzessionsreihen, die Herausbildung der Graphen der artspezifischen Matrix erfolgte mittels Clusteranalyse. Abiotische Variablen und Spezies-Korrelationen in der Aufeinanderfolge innerhalb der Faunengebiete erfolgten mittels Hauptkomponenten-Analyse. Diese Untersuchungen erfolgten auf der Grundlage der Arbeiten von PODANI mit Hilfe des von ihm entwickelten Programmpakets. Die Arten- und Biotop-Analyse erfolgte mit Hilfe der Clusteranalyse, womit die ökologischen Artgruppierungen getrennt werden können. Regressionsanalysen erfolgten zwischen Faunenkreis und Klima-situation, Rangkorrelationen wurden zur Analyse der Geschiebefauna durchgeführt, der  $\chi^2$ -Test fand Verwendung bei der Trennung der Faunenkreise.

Neben den ökologischen Artgruppen, erfolgte eine Biotopeinteilung und Biotop-Typisierung, in die neben Ernährungstyp-Einteilungen auch Untersuchungen über saisonale und fluktuative Faktoren mit einbezogen wurden. Die Zusammensetzung von Pflanzen-gesellschaften und der Einfluß auf die Schneckengemeinschafts-Sequenzen werden diskutiert. Zwei Wald-Sukzessionsserien aus der Ungarischen Tiefebene wurden nach dem Graphen-Verfahren mittels Pascal-Algorithmus rechentechnisch ausgewertet. Die Spitzen-Stabilisation wurde nach GALLOPIN errechnet. Bezüglich der Schneckenvermehrung sowie über den Laubabbau wurden Wachstums- und Nahrungs-verbrauchs-Untersuchungen durchgeführt.

## Zusammenfassung der Ergebnisse

### *Tiergeographische Neugliederung*

Mittels arealanalytischer Verfahren gelang es, 139 in Ungarn nicht synanthrop vorkommende Landschneckenarten nachzuweisen. Weiterhin gelang es mit diesem Verfahren, 23 Faunenkreise 10 Verbreitungsräumen zuzuordnen. Im Unterschied zu anderen auf dem Festland lebenden Tiergruppen gehören zur Landschneckenfauna auch fossile Vorfahren. Zieht man die Entstehungsgeschichte des Karpatenbeckens in die Überlegungen mit ein, läßt sich ein eigenes dynamisches paleoklimatisches Verhalten in den einzelnen Faunenkreisen erkennen. Diese Faunenkreise sind an glaziale, interglaziale bzw. interstatale Abschnitte trockener bzw. feuchter Perioden sowie an preboreale und boreale Zeitabschnitte des Atlantiks gekoppelt. Boreo-montane Disjunktionstypen spielen als Arten in den Schneckengemeinschaften nur eine akzessori-



sche Rolle. Im europäischen Pliozän vorkommende Arten dominieren auch gegenwärtig in unseren Flußniederungen.

Im Monat Juli wird auf Grund des bestehenden Feuchtigkeitsminimums, über eine Zeitdauer von 14 Stunden ermittelt, ein Mittelwert entsprechend geographischer Landschaftseinheiten ausgewiesen und je nach Art ein Wert mit zugehöriger Abweichung als Korrelation errechnet; ein negativer Korrelationskoeffizient ist mit kontinentalen, ein positiver Wert mit subatlantischen Faunenelementen auf einem Irrtumsniveau von  $p = 0.1$  bis 5% verbunden. Die rezenten Klimazeichen der Faunenkreise machen auf der Grundlage aktueller Kriterien wahrscheinlich, daß sie als Refugien dienen können, mittels derer man auf frühere Klimaverläufe schließen kann. In Einklang mit den Grenzen gegenwärtiger Verbreitungstypen gibt es solche Klimatypen, die ein kühles-mildes und verbreitetes mittleres, trocknes Kontinentalklima bevorzugen und sie gestatten eine Trennung von jenen Typen, die ein warmes-kontinentales subatlantisches Klima bevorzugen; so gestatten diese Arten eine Differenzierung der zu trennenden Faunenkreise. Auf der Grundlage gemeinsamer Verbreitungsmodi der Pflanzen wird die Nützlichkeit des areal-analytischen Verfahrens hervorgehoben. In der ungarischen Schneckenfauna spielen vor allem ponto-mediterane, sibirisch-asiatische, mitteleuropäisch-montane und holometiterrane Faunenkreise eine größere Rolle.

#### *Faunistische Ergebnisse*

In der Ungarischen Tiefebene kommen 104 Arten vor. Der größte Teil von ihnen sind Bewohner der Waldgesellschaften und der Flußtäler. Im Bükkgebirge leben 100 verschiedene Arten. Eine faunistische Seltenheit ist eine für Wiesen typische Art die Feuchtigkeit, Schluchten- und Bergwälder bevorzugt und die klimazonalen Waldgesellschaften zugeordnet werden kann.

#### *Die Verteilung der die Arten beeinflussender Faktoren*

Neben der Artenaufteilung spielen in den naturgeographischen Einheiten historische sowie klimatische, bodenkundliche und fluviatile Ursachen eine Rolle. Unter den klimatischen Faktoren wurde der Einfluß von mikro-, meso- und makroklimatischen Auswirkungen untersucht. Dabei wurden zur Beurteilung des Faunenbestandes faunistische, ökologische und tiergeographische Verfahren herangezogen.

Im Bükkgebirge lassen sich auf Grund intrazonaler und zonaler Vegetationsgesellschaften die Schneckenarten in 2 Gruppen einteilen: die der Wärmeperioden (Pr, B) und die mit atlantischem Ursprung. Beide Kurven verlaufen zueinander komplementär. Die komplementäre Ausbildung verdeutlicht im Falle des Bükkgebirges die Faunengeschichte und die zonale Waldzugehörigkeit, zwischen beiden Artengruppen gehören die preborealen-borealen Arten zu 4 ökologischen (xeromesophilen) Artengruppen, die atlantischen Arten verkörpern die 1-2 (hygrophilen, mesohygrophilen) ökologischen Artgruppen.

Im Bükkgebirge sind auf Grund der Klusterverteilung die zonalen und intrazonalen Schneckenbestände an die planare Eichenzone und an die collin-montanen intrazonalen Gesellschaften gekoppelt. Hierbei spielt die Inklination submediterranen Klimas eine



Rolle. In den Zerreichenwäldern des Bükkgebirges ändert sich die Individuenzahl und die Abundanz periodisch und die Nieder-schlagsmengen lassen deutlich einen 4 - 5 jährigen Rhythmus erkennen. Eine Senkung der Abundanz ist mit einer zunehmenden Zahl schneckenfressender Räuber korreliert.

In der Ungarischen Tiefebene sind auf Grund der Entstehungsgeschichte kontinentale Faunenkreise erstrangig. Vor der Entwässerung waren sibirisch-asiatische, aus preboralen-boralen Faunenkreisen stammende Faunenelemente aus Sand- und Lößwiesen kaspischen und turkestanischen Ursprungs typisch. Daneben spielt ein Faunen-transport durch die Flüsse eine Rolle, wobei das gegenwärtige Klima und hydrologische Parameter die rezente Einwanderung limitieren. Der Faunen-transport durch Flüsse wird durch 48 begrenzende Artaufgliederungen im Über-schwemmungsgebiet der Flüsse belegt.

Neben den Flüssen spielen für die Artenverteilung die mittlere Transport-kapazität der Ströme eine Rolle. Von 48 Arten gelangen 24,9 % durch eine Wald-vermittlung in die Ungarische Tiefebene. Die aus dem Donau-Theiß-Staugebiet stammenden Faunenkreise belegen einen solchen Faunentransport (alpin-karpato, boreo-alpin, bzw. karpatisch und karpatisch-sudetisch). Dies wird sowohl durch neuzeitliche aber auch durch die Rangfolge fossiler Elemente der Flußgeschiebe belegt. Die individuellen Flußgeschiebe und initialen Gesellschaften der Uferregionen sind in ihrer Rangfolge mit jenen der Theiß vergleichbar.

Auf Grund von Untersuchungen zum Mikroklima wird eine gelegentliche und ständige Ansiedlung im Uferbereich vom Neigungswinkel, der West-Ost-Lage, dem dadurch beeinflussten Feuchtigkeitsgehalt, dem Verdunstungsgrad, der Ufererosion sowie Anlagerungs- und Ausschwemmungserscheinungen beeinträchtigt. Einbürgerungsvorgänge werden durch das feuchte Klima im oberen Theißbereich begünstigt. Während der Überschwemmungen ziehen sich die Schnecken in den Boden oder in den Luftraum zwischen den Wurzeln des Uferbereichs zurück. Zwei Wochen nach dem Hochwasser werden die ursprünglichen Verteilungen wieder hergestellt. Laut saisonaler Untersuchungen außerhalb des Inundationsraumes der Gras- und Waldbiotope ist die Bodenfeuchtigkeit einer der begrenzenden Faktoren. Unter den einzelnen naturgeographischen Landschaftseinheiten entstehen in der Verteilung der Arten wegen Unterschieden hinsichtlich des Wärmehaushalts genetisch determinierte bodenständige Typen, was bereits aus Arbeiten von HORVÁTH für das Pleistozän der Ungarischen Tiefebene geschlußfolgert werden kann.

Auf Grund gegenwärtiger Daten dieser Klimakreise lassen sich Befunde über Schnecken mit den Daten der Klimagebiete mit Hilfe der  $\chi^2$ -Probe untersuchen, wobei einerseits ein trocken-warmer (A1-2; Crisicum, Prematricum), ein moderat-warmer (B4, A4, B1) Klimabereich (Colecense, Titelicum, Samicum, Nyirsegense) zu erkennen ist und eine Verteilung der Schnecken den Klimazonen entsprechend vorgefunden wird. Trocken-warmes Klima erweist sich dabei für eine Expansion als der begrenzende Faktor. Das andere vorkommende Ordnungsprinzip der naturgeographischen Landschaftseinheiten ist die geographische Faunenkreisordnung. Ein Ergebnis dieser

Zuordnung zu den tiergeographischen Faunenkreisen ist, daß sich auf Grund der aus den Karpaten kommenden Flüsse die Landschaftseinheiten der Ungarischen Tiefebene von denen der Donau und der Drauebene unterscheiden. Laboratoriumsuntersuchungen bezüglich des Nahrungs-bedarfs von Schnecken aus dem Bükkgebirge und der Ungarischen Tiefebene machen wahrscheinlich, daß in trockenem Klima der Substanzabbau niedriger (5 %) und in feuchtem Klima höher (40 %) ist.

#### *Zusammenhang zwischen den Arten und abiotischen Faktoren*

Für beide Landschaftseinheiten wurden die Einflüsse abiotischer Faktoren auf die Arten untersucht. Mit Hilfe der Methode von FEOLI und ORLÓCZI wurden die ökologischen Artgruppen je nach Individuenzahl der Einzelart assoziiert und mit abiotischen Faktoren korreliert. Im Bükkgebirge waren es hygro-mesohygrofile Arten (1-2 Artengruppen) die mit der Meereshöhe (Temperatur, Feuchtigkeit) und der Flußwasserdichte, von den mesoxerophilen Arten (4 Artengruppen) jene, die mit dem Neigungswinkel, den Arten der Uferzone (3 Artengruppen) jene, die mit den Werten der Quellgewässer korrelieren.

Die an offenes Gelände gebundenen Arten in der Ungarischen Tiefebene (D) korrelieren mit dem pH-Wert, der Uferzone, die der Sumpfwälder (E, B) mit hydrologischen Werten, mit der Feuchtigkeit, die der C-Gruppe mit dem Klima und dem Alter des Waldes, die schattenliebenden der A-Gruppe mit dem Kronenschluß und dem Klima, und es gibt eine Korrelation zu hydrologischen Graden. Es gibt 5 ökologische Artengruppen (D, E, B, C, A). Die der offenen (D) Landschaft, die der Uferzone (E, B), die des Waldes (A, C), eingeteilt nach ihrer Häufigkeit. Die häufigsten Arten korrelieren miteinander.

#### *Regionale Zusammenhänge.*

Die Zusammenhänge zwischen der Schneckenfauna aus klimazonalen Eichenwäldern von 36 Sammelplätzen wurden anhand einer nach Faunenkreisen geordneten Artenliste untersucht. Entsprechend einer Cluster- und Haupt-komponenten-Analyse werden die Arten aus den europäischen Eichenwäldern 3 Untergruppen zugeordnet: der westlich-paleoarktisch-mediterranen Landschaft, dem atlantischen Faunenkreis (Südschweden, Herzyn-Gebiet, den Mittelgebirgen, den Berner Alpen) sowie den Nordkarpaten und dem Bükkgebirge, das auch das mittlere Donau-Faunengebiet mit einschließt. Die hier vorkommenden Faunenkreise dominieren in diesen Arealen mit mehr oder weniger hohen Zahlen in Abhängigkeit vom Verbreitungszentrum und der durchschnittlichen Entfernung zum Mittelpunkt.

#### *Schneckengemeinschaften beeinflussende Faktoren.*

Entsprechend der Pflanzenzöologischen Literatur des Bükkgebirges und der Ungarischen Tiefebene wurden die zugehörigen Sukzessionsserien untersucht: im Bükkgebirge die Uferpartien, Kalkwiesen und vulkanischen Wiesengesellschaften bis hin zum Buschwald, zonale und intrazonale Waldgesellschaften dieser Klimazone. In der Ungarischen Tiefebene waren es die Sukzessionsreihen der sandig-organogenen und mineralogenen Serien.



Die Sukzessionsstadien der Schneckengemeinschaften stimmen laut Kluster- und Hauptkomponenten-Analyse mit den in der Literatur beschriebenen Pflanzen-zönologien überein. Dabei decken sich die Mittelwerte der Sukzessionsreihen mit dem Temperatur-Niederschlags-Index und mit der mittleren Streuung des Wachstumsortes, und die Folgen von Drainage und Waldbaumaßnahmen werden sichtbar. Die Schneckenpopulationen der Ungarischen Tiefebene und des Bükk-gebirges werden auf der Grundlage der Ergebnisse von speziellen Bodenuntersuchungen einem mittleren Gradienten der Feuchtigkeitsdurchschnitte am Wachstumsplatz zugeordnet. Hierbei spielen die unmittelbare Bodenfeuchtigkeit und im Bükkgebirge der Bodensatz eine Rolle.

### *Strukturelle Charakteristika der Schneckengemeinschaften*

Die untersuchten Schneckengemeinschaften des Bükkgebirges und der Ungarischen Tiefebene weisen in den Sukzessionsreihen Schneckengemeinschafts-Charakteristika auf (eine mittlere Konstanz-Subkonstanz akzessorischer Arten,  $A/m^2$ , und  $H'$  Meßwertverteilungen und eine Verhältnisverteilung der Artengruppen), deren Charakteristika eine wachsende Tendenz aufweisen. Mit Ausnahme der unter menschlichem Einfluß stehenden (Tourismus, Waldwirtschaft) Grasland- und Waldkulturen, treten eine Verringerung der Diversitas und Abbundanz auf und die akzessorischen Elemente nehmen zu. Gleiche Erfahrungen liegen für die in der Ungarischen Tiefebene gelegenen Waldpflanzungen vor. Die strukturellen Charakteristiken lassen sich so für eine Indikation von Kultureinflüssen heranziehen. Anzeichen für die unter Siedlungs- und Waldbewirtschaftung stehenden Bereiche sind Verringerung der Artenzahl sowie eine Abnahme früher Sukzessionsreihen. Die einzelnen Artengruppen verändern sich in ihrer Aufeinanderfolge sowie auch innerhalb der Sukzessionsreihen beträchtlich.

Auf Kalkstein und vulkanischem Untergrund erfolgt im Verlauf der Bewaldung Strauchwald-typischer Areale mit Steppenelementen ein prozentuales Anwachsen der Strauchwaldbewohner, für Sukzessionsreihen der Uferbereiche und zonalen Wälder ist eine Zunahme der Waldbewohner typisch. Unter den einzelnen ökologischen Artengruppen sind für Kalk- und Vulkangestein xeromesophile Arten, für die Uferzonen und zonalen Wälder die 1-3 Feuchtigkeitsgrade liebenden Arten sowie xeromesophile Vertreter mit komplementären Anteilen typisch. Kultureinfluß begünstigt das Zunehmen ubiquitärer Artengruppen.

In der Ungarischen Tiefebene nehmen in den Sukzessionsreihen parallel mit zunehmender Trockenheit oder Feuchtigkeit die Artenzahl zu, die Diversität und die Zahl der häufig vorkommender Arten steigt an. Unter den einzelnen ökologischen Artengruppen sind es in der sandigen Sukzessionsreihe (D) vor allem Arten der offenen Landschaft, in organogenen Sukzessionsreihen E, B, C (Uferregion, Sumpfbewohner, lichtliebende subhygrophile) Arten, in mineralogenen Sukzessionsreihen (E, C), die eine dominante Konstanz aufweisen. Im räumlichen Verlauf der Sukzessionsreihen verändern sich die Artengruppen in komplementärer Weise. Bei Sandbodenreihen sind dies die A, C -Artengruppen und die untergeordnete D-Gruppe (Schatten- oder Lichtliebend, mit Feuchtigkeitsansprüchen), bei den organogenen Sukzessionsreihen die E



und D-Gruppen, bei den mineralogenen Reihen die A- und C-Gruppen, die eine komplementäre Veränderungstendenz erkennen lassen.

Vergleichbare komplementäre Veränderungen zeigen sich auch je nach Biotop- und Ernährungstyp. Bei der Sandreihe sind die Steppenbewohner (S-) und die Bewohner der Strauchregion (BE-Gruppen) komplementär, anderenorts sind es omnivore (O) und herbivore Arten (H-), die im komplementären Verhältnis stehen. Bei den organogenen Sukzessionsreihen sind es die VP-Gruppe (Uferregionen), sowie die BE-, O- und die H-S-Artengruppen (omnivor, herbivor, saprophag), bei den mineralogenen Sukzessionsreihen sind die VP-, BE- und E-Gruppen (Waldbewohner) für die eine Zunahme der O- bzw. eine Verringerung der H-Gruppen typisch ist.

Waldwirtschaft und Siedlungseinflüsse, Weidewirtschaft und Mähvorgänge beeinflussen die Dominanz der Sukzessionsreihen. Bei Sandreihen sind es vor allem die D- und S-Gruppen, im Siedlungsbereich die herbivoren Arten. Heu- und Weidewirtschaft in den Graslandkulturen begünstigen die Ausbreitung saprophager Arten. Bei den organogenen Reihen dominieren im Siedlungsbereich vor allem Steppenbewohner und omniphore Arten. Bei mineralogenen Reihen herrschen im Siedlungsbereich vor allem die Steppenbewohner (S) und die Typen mit omnivorer Ernährungsweise vor.

#### *Auswertung der Sukzessionen mittels Graphen*

Die Sukzessionsstadien der Sand- und Mineralreihen werden mittels graphischer Auswertung (Pascal-Algorithmus) beurteilt. Die einzelnen Wiesen- und Waldgesellschaften liegen auf Graphenniveau, die Subassoziationen sind in Spitzenwerten sichtbar. Der Algorithmus wählt auf dem Niveau von 5 % die charakteristischen Wege aus und es erfolgt eine Analyse der Strukturelemente.

Die Sukzessionswege sind kürzer und verlaufen mit 2-5 Spitzenwerten. Dabei ist eine Wachstumstendenz charakteristisch, der Verlauf ist nicht linear, sondern verzweigt in Richtung Feuchtigkeit oder Trockenheit. Ein Teil der Charakterarten verbleibt in der Sukzessionsreihe ständig im oberen Bereich. Dem Graphenniveau folgend wächst die Stabilität. Im Verlauf der Sukzessionsreihen der Schnecken-gemeinschaften scheint sich das Sukzessions-Toleranz-Modell zu bestätigen. Ähnliche Befunde ergaben sich auch beim Austausch der Charakterarten in Skandinavien bei der Besiedlung einer Insel.

#### *Tiergeographische Aufteilung zwischen den Sukzessionsreihen*

Für die Ungarische Tiefebene sind für die drei Sukzessionsreihen die Faunenkreisaufteilung und die Veränderungen in den Sukzessionsreihen als vergleichbare ökologische Charakteristika typisch. Kennzeichnend für die Faunenkreise sind dominant charakteristische Elemente. Für den Bereich der mineralogenen Reihen sind es 18 Faunenkreise, den anderen zwei Sukzessionsreihen stehen 12-12 Faunenkreise gegenüber. In extrazonalen Sandreihen dominieren kontinentale ponto-pannonische und sibirisch-asiatische Faunenkreise und diese sind dominant und komplementär zu holo- und ponto-mediterranen Faunenkreisen. Anthropogene Einwirkungen gehen zu Lasten kontinentaler Elemente wobei der Anteil holo-mediterraner Arten zunimmt.

Für organogene Reihen sind kontinentale Faunenkreise (ostsibirische, holarktische) typisch. Mit der Kronenschließung von *Fagion illyricum* stellt sich ein wichtiges Element ein. In Siedlungsgebieten nimmt der Anteil des holarktischen Faunenkreises zu. Bei mineralogenen Sukzessionsreihen ist die Zusammensetzung des Faunenkreises infolge der Wirkung des Flußfaunatransports reicher. Typische Faunenkreise sind der ostsibirische, der holarktische, der ponto-adriatisch-holo-mediterrane und der karpato-sudetische Teil. In Siedlungsgebieten nimmt infolge holarktischer Elemente eine Kontinentalisierung zu. Die einzelnen Sukzessionsreihen spiegeln in ihren Faunenkreisen ihre Geschichte wider. In den preboralen-boralen Sandgebieten weisen kontinentale, ponto-kaspische, turkestanische, ponto-pannonische Elemente auf ihre Herkunft aus preboralen-boralen Faunenkreisen der Steppe hin. In den mineralogenen Reihen weisen zum großen Teil subatlantische Elemente auf die Herkunft vom Atlantik hin. Die Existenz dieser Faunenkreise, bzw. ihr Fehlen in den Sukzessionsreihen, sowie Unterschiede zwischen den Landschaften (bodenkundliche, orographische, hydrologische, waldmäßige, klimatische, faunen-geschichtliche) erweisen sich als brauchbare Indizien, um natürliche und anthropogene Faktoren zu differenzieren.

### Möglichkeiten des Nachweises menschlicher Einwirkung

Das Vorhandensein einer Empfindlichkeit gegenüber den vielfältigen abiotischen Faktoren der Umwelt zeigt sich auch in den einzelnen Biotopen wobei das Material, das aus absoluten Sammelmethode stammt, und insbesondere die gebietsmäßige Zusammensetzung der wenig beweglichen Bodenschnecken, als Index für Veränderungen aus deren begrenzten Lebensraum dienen kann. Die Erfassung von Umweltfaktoren erfolgte auf vielfältige Weise. So ist das Verschwinden der wenig toleranten Arten in der Ungarischen Tiefebene und im Bükkgebirge eine Begleiterscheinung anthropogenen Einflusses. Die Artspezifität der Wachstumskurve einzelner Arten sowie deren phenotypischen Veränderungen (Bänderung, Farbe) spiegeln Einflüsse auf den Feuchtigkeitzustand des Biotops wider.

Die Aufteilung der Populationen (Untersuchung von 100 Quadraten in den Abmessungen von je  $10 \times 10$  Einheiten) wurde in den meisten Biotopen saisonal, im Frühjahr und Herbst inequal-kumulativ, mit Pausen in der Sommerzeit inselmäßig in nicht entwässerten Lebensbereichen durchgeführt. Drainage, Kronenschluß, Lichtverhältnisse und Bodenfeuchtigkeitsänderungen beeinflussen die Verteilung mit inselmäßiger Verringerung der Diversität. Die Mittelwerte für lebende und tote Individuen ergaben sich auf der Basis einer 50 % igen Abweichung, wobei die Wirkung von Luftverunreinigungen im Straßengrabenbereich im Engpaßtal von Szádelő aufgezeigt werden konnte bzw. über Windeinwirkungen in den einzelnen Waldtypen der Spitzenlagen des Bükkgebirges, Ost-, Ost-West-, und Westwind. Bekannte anthropogene (Drainage, Schutzwald, Auslichtung, Waldwirtschaft) und natürliche Einflüsse (Hochwasser, *Lymantria* Gradation) auf die Fluktuation zeigen sich bei saisonalen Untersuchungen in drei der derzeit geschützten Gebiete. Auf Grund der seit mittlerweile



ca. 15 Jahren erfolgenden Probesammlungen läßt sich feststellen, daß fortschreitende Drainagemaßnahmen Ursache für die Artenzunahme biotopfremder Individuen in bestimmten Arealen sind. Die Auswirkungen der sowohl in feuchtem als auch in trockenem Klima vorgenommenen Waldbaumaßnahmen (der Ortschaften Bagiszeg und Landor) führen in trockenem Klima zu einer Eutrophisierung des Bodens (z. B. in Landor). Tourismus und Heuwirtschaft verursachen bei Bergwiesen einer Verringerung und Homogenisierung der Arten. In der Ungarischen Tiefebene verursacht Heuwirtschaft eine Vermehrung saprophager Arten und führt zur Eutrophisierung. Sukzessionsreihen der verschiedenen Altersgruppen waldbevorzugender und der zum Wald gehörenden Arten und die Diversität nehmen ab. Die Auswirkung der Beweidung und des Anbaus mit starkwachsenden Pflanzen besonderer Arten führt im Vergleich zu Kontrollflächen zu Subassoziationen wie sie sonst nur auf Sandbodenweiden und in Auwäldern anzutreffen sind, wobei sich eine Abnahme der Charakterarten von ca. 25-88 % bemerkbar macht, die Artenzahl, die Individuenzahl, der  $A/m^2$ -Wert, die Diversität, die Zahl der Jugendstadien und des Feuchtigkeitsgehaltes des Bodens gegenüber der Vergleichsflächen ist verringert.

### Schlußfolgerungen für den Naturschutz

Um die anthropogenen Einflüsse im Bükkgebirge und in der Ungarischen Tiefebene zu verhindern, die sich sowohl im Bükkgebirge als auch in der Ungarischen Tiefebene hier bisher gezeigt haben, sind Veränderungen in der Behandlung der Naturschutzgebiete notwendig. In der Ungarischen Tiefebene ist dabei die vorrangige Frage, wie eine Korrektur des Wasserabflusses zu erfolgen hat. Bei Wiesenflächen der Bergregion und in der Ungarischen Tiefebene sind während der Vermehrungszeit der Schnecken im Frühjahr, im Interesse der Feuchtigkeits- und Schattenliebenden Bodenfauna, Mähprozesse zu verringern bzw. ganz zu unterlassen. Die intensive Waldwirtschaft in Naturschutzgebieten ist zu beenden. Die Gesundungsprozesse im Wald sind unter Aufsicht der Naturschutzbehörden durchzuführen. Die altherwürdigen Baumbestände an den Bachläufen der Gebirge, die stationären und zonalen Waldgesellschaften in Gebieten mit sehr dünner Bodendecke bedürfen des besonderen Schutzes, die zugehörige Fauna muß der Wiederbesiedlung der Stammgebiete dienen. Muffelwild hat aus den intrazonalen Felswäldern und aus den Nationalparks zu verschwinden.



Summary of thesis submitted for the degree of Candidate of Science

REPRODUCTIVE STRATEGIES AND SPATIO-TEMPORAL PATTERNS OF  
TERRESTRIAL ISOPOD POPULATIONS

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Introduction

Woodlice (**Isopoda**, **Oniscidea**) are the most successful invaders of terrestrial habitats among **Crustaceans**. This group of animals is worth for special attention in both biogeographical and ecological respects. Although their dispersion abilities are highly limited, they are widely distributed: their occurrence involves all kind of habitats from the sea through sea shores into the main land, some species are adapted even to desert circumstances. **Isopods** being a polyphyletic group have diverse life-history strategies adapted to life on land. Different structural, morphological, physiological and behavioural adaptations help them in possessing the mentioned wide variety of habitats. The most important among them are the development of brood pouch and pseudotracheae which mean high protection for their offspring and the possibility of air respiration, respectively. They also have a wide range of behavioural adaptations: they are able to follow the changes of the most important environmental factors by their diurnal, and seasonal rhythm. Their within habitat distribution is changing from time to time in accordance with environmental pressure. Humidity is of crucial importance in choosing their habitat. The existence of necessary humidity is the key limiting factor, more important than any other environmental factors (e.g. food resources, temperature).

Reproduction is perhaps the most important element of their diverse adaptation of life style. The timing, frequency of reproduction, the rate of reproducing females are varying among species within their iteroparous or semelparous reproduction strait. This variety which appears very often within species, among habitats, between different years within the same population, respectively, means a high plasticity.

The role of woodlice within biological communities stays in their decomposing activity. They feed generally on dead organic material, first of all on detritus. Their assimilation efficiency is rather low, their importance stays not really in the decomposition of dead plant material but in the exposure, breakdown or microorganism inoculation of that.

Woodlice appear practically in all habitat types due to their mentioned wide adaptation abilities. They can be found in cryptozoic microsites within the given habitat. To avoid desiccation — as main stress — they aggregate in so called shelter sites of high humidity.

### Aims of the study

Three main problems were raised in the study: All of these concerns events on population level and their possible background factors on different scales.

(1) The first group of questions regarding the inner mechanism of populations:

- can we determine the actual reproductive potential of different species;
- is it possible to quantify the decrease in the number of offspring during the different phases of reproduction;
- are the intra- and interpopulational reproductive differences in connection with any environmental factor;
- what is the background mechanism of these population processes staying in?

(2) The second group of questions concern the dynamics of populations and communities:

- how can we describe the dispersion of certain isopod populations within a habitat, by which factors is it determined in the different geographical regions;
- how does the spatial distribution of populations depend on the heteromorphy of habitats;
- what is characteristic for population densities in the investigated habitat-types;
- do the certain population characteristics change in time (e.g. sexual rate, age group distribution, number of offspring);
- what is the composition of isopod communities look like at larger scale, in inter-habitat comparison;
- what are the qualitative and quantitative relations of communities determined by;
- is there any dependent relation between species, are there any real isopod assemblages?

(3) The third group of questions concerns the assemblages of macrodecomposer animals.

The next problems were raised on the basis of sampling in 29 habitats of Kiskunság region:

- is it possible to characterize the habitats by the composition of their decomposer fauna;
- can the habitats of same character be concentrated into groups on the basis of their similarity;
- how do the members of communities reflect the possible habitat heteromorphy?

### Study sites and methods

In the frame of diversity monitoring and state assessments samplings were done on 29 sites in the southern part of the Great Hungarian Plain (Kiskunság, Békés regions) and in the Mediterranean region (North-Israel), respectively. These samples provided data not only for isopods but also for faunistic characterization of other epigeic macrodecomposer groups (Diplopods, Gastropods).



The phytocoenoses studied were all nature close, most of them under nature protection. In the case of three, typical lowland habitats regular samplings (for 1 or 2 years) provided the basic data sets.

Among sampling methods there were both pit-fall trapping, grid sampling and soil sample extractions corresponding to the particular aims. For evaluation of data sets different statistical methods, population and community characteristics were used: similarity evaluations by clustering (based on CHEKANOWSKI and JACCARD indices), computing correlations,  $\chi^2$  probe for testing the results and to investigate the species associations; SHANNON-WIENER diversity index and dispersion index for characterization of communities and spatial distribution of populations, respectively.

Female individuals were dissected to state reproductive characteristics, that is number and developmental stage of oocytes, embryos and manca. The field data of three common species (*Trachelipus nodulosus*, *T. rathkei* and *Armadillidium vulgare*) were used for comparison of their reproductive periods.

The effects of photoperiod and temperature on *Porcellio ficulneus* were studied under laboratory conditions.

### Summary of new scientific results

#### *Reproduction*

(1) The number of oocytes before vitellogenesis that is during a "resting" stage was stated in the case of 14 populations of 11 species using the technique of dissection. It was proved that the number of oocytes is increasing in accordance with the females' size not only within but also among species.

(2) Data were presented for the actual reproductive potential of isopods. From the number of oocytes in the ovary we can deduct to the number of future offspring. Under field conditions 80% of oocytes develop into fit descendants.

(3) The recognition and description of oosorption, the determination of its rate is new for the literature in the case of isopods. It was proved convincingly by experiments that the effects of environmental factors may be effective through the process of oosorption. Oogenesis does not start under certain light/temperature conditions (here: 10 °C/ 10 h light). Both high temperature and prolonged light decreased significantly the length of the different stages of reproduction, but it caused the significant decrease of offspring numbers in all cases.

#### *The structure and spatio-temporal changes of isopod populations*

(1) The temporal fluctuations among years, the changes in abundance, spatial dispersion within habitats, changes in age structure and sex ratio of populations and their possible background factors were characterized. Both fluctuations, spatio-temporal and density changes are in accordance with microclimate fluctuations influenced by macroclimate. The size of shelter sites is also fluctuating being smallest during the most extreme summer period when the dispersion of woodlice within their habitat is usually highly aggregated.

(2) The seasonal spatial density replacements in a habitat and a possible diurnal migration of isopods among habitat patches was not mentioned before in the literature.

(3) It was stated that the sites of aggregation under our climatic conditions are marked by the size and placement of shelter sites differently to the "best quality food" theory under more equalized climate.



(4) The macrodecomposer taxa with epigeic activity (Gastropoda, Isopoda, Diplopoda) reflect environmental heterogeneity on the same scale as vegetation but on a different way: while isopods and diplopods indicate heterogeneity by their individual numbers, gastropods change also their species composition.

*The distribution of terrestrial isopods at different scales*

(1) By scale of distribution we may speak about "minidistribution", the distribution of certain species and their individuals within habitat patches, shelter sites;

(2) "microdistribution" means the dispersion of species within a habitat;

(3) "macrodistribution" is the distribution of species among similar and different habitat types within a geographical region;

(4) and the geographical scale what belongs already to the zoogeographycal categories. From ecological point of view the first three scales mean an operative method. The within-population processes need mini-, or micro-scales, while inter-population comparisons need a macro one.

## CHRONICLE

### Personalia

DR. LÁSZLÓ ERDEI (Department of Plant Physiology), DR. JÓZSEF TOLDI (Department of Comparative Physiology) and DR. BÉLA MATKOVICS (Biological Isotope Laboratory) have been appointed Professors by the President of the Hungarian Republic.

MRS. DR. ISTVÁN SZÖLLŐSI (Biological Isotope Laboratory) has been appointed Ass. Prof. by the Rector of J. A. University.

DR. KORNÉL KOVÁCS, Senior Research Associate at the Biological Research Center of the Hungarian Academy of Sciences in Szeged, has been appointed Ass. Prof. by the Rector of J. A. University.

Ass. Prof. DR. MAGDOLNA ÁBRAHÁM (Department of Biochemistry), Prof. DR. LÁSZLÓ ERDEI (Department of Plant Physiology), Ass. Prof. DR. KORNÉL KOVÁCS (Department of Biotechnology) and Ass. Prof. DR. ERZSÉBET MIHALIK (Department of Botany) have been appointed to the chairs of those Departments by the Rector of J. A. University.

Prof. DR. LAJOS FERENCZY, head of Department of Microbiology, has been appointed Ordinary Member of Hungarian Academy of Sciences in 1995 and deputy chairman of Biological Section of the Hungarian Academy of Sciences in 1996.

From May 1995 Prof. DR. MIKLÓS KEDVES is an active member of the New York Academy of Sciences.

### Retiring

Prof. DR. OTTÓ FEHÉR (Department of Comparative Physiology), Ass. Prof. DR. SÁNDOR GULYÁS (Head of Department of Botany), Prof. DR. MIKLÓS KEDVES (Department of Botany), Ass. Prof. DR. IMRE MARÓTI (Department of Botany), Ass. Prof. DR. IMRE MÉCS (Head of Department of Biotechnology) and Prof. DR. FERENC ZSOLDOS (Head of Department of Plant Physiology) retired in 1996.

### Awards

Prof. DR. OTTÓ FEHÉR, Prof. DR. FERENC ZSOLDOS, Ass. Prof. DR. SÁNDOR GULYÁS and Ass. Prof. DR. IMRE MARÓTI have been awarded the medal "For the Time of Pedagogical Service" by the Minister of Culture and Education.

The council of József Attila University honoured Prof. DR. FERENC ZSOLDOS, retired university professor, earlier head of the Department of Plant Physiology, with the title of "Professor Emeritus".

The Hungarian Biological Society awarded the "Jávorka Sándor prize" to DR. GYÖZÖ CSONGOR, retired honorary associate professor.

### Habilitation proceedings

In 1995-96, the following application lectures for habilitation were presented at the Biological Section of the Faculty of Natural Sciences:

Biological Isotope Laboratory:

Prof. DR. BÉLA MATKOVICS: Oxygen radicals and antioxidant systems.

Cell Biological and Evolutionary Micropaleontological Laboratory of the Department of Botany:

Prof. DR. MIKLÓS KEDVES: Phylogeny and paleophytogeography of the angiosperms on the basis of palynological results.

Biological Research Center of Hungarian Academy of Sciences in Szeged:

DR. ÁRPÁD PÁRDUZ: Plastic nervous changes in response to hormonal effects.

Department of Botany:

Ass. Prof. DR. SÁNDOR GULYÁS: Primer attractants (nectar, pollen), biological importance of bloom and pollination.

Ass. Prof. DR. MIKLÓS JUHÁSZ: Palynology of earliest angiosperms.

Department of Comparative Physiology:

Ass. Prof. DR. JÓZSEF TOLDI: The plasticity of the somatosensorial system.

Ass. Prof. DR. MAGDOLNA SZENTE: Normal and pathological nervous activity in the cortex.

Department of Genetics:

Ass. Prof. DR. PÉTER MARÓI: An efficiency investigation of moulting hormones in *Drosophila*.

Department of Plant Physiology:

Ass. Prof. DR. LÁSZLÓ ERDEI: The sensation and transmission of signs in plants.

Department of Zoology and Cell Biology:

Ass. Prof. DR. ÉVA FEKETE: The embryonal evolution of the intestinal nervous system.

Ass. Prof. DR. KÁROLY GULYA: Peptideric neurotransmission.

Ass. Prof. DR. KATALIN HALASY: Morphology and synaptic connections of basket cells and dendrite targeting interneurons in the rat hippocampus.



### Scientific degrees

The degree of Doctor in Biological Sciences has been awarded to:

Ass. Prof. DR. LÁSZLÓ GALLÉ (Department of Ecology), with a dissertation: Organization of ant communities.

The degree of Candidate in Biological Science has been awarded to:

DR. IRMA TARI (Department of Plant Physiology), with a dissertation: The role of ethylene in the growth and development of bean seedlings treated with the growth retardant paclobutrazol.

DR. LÁSZLÓ KÖRMÖCZI (Department of Ecology), with a dissertation: Spatio-temporal patterns and pattern transformations in sand grassland communities.

DR. ERZSÉBET HORNUNG (Department of Ecology), with a dissertation: Reproductive strategies and spatio-temporal patterns of terrestrial isopod populations.

The degree of Doctor Philosophy (PhD) has been awarded to:

DR. SÁNDOR TÓTH (Department of Biotechnology), with a dissertation: Influencing the antiviral activity of interferons by amino acids.

### Conferences

International Orthodontic Congress organized by the Hungarian Orthodontic Society, the Committee of Dentistry and Oral Surgery of Academic Board of Szeged, the Clinic of Dentistry and Oral Surgery of Albert Szent-Györgyi Medical University and the Department of Anthropology, József Attila University was held in Szeged 5-8th September, 1996. The congress consisted of two sessions: Orthodontics; Facial Clefts and Syndromatology. The Congress was preceded by a training course on the "Anthropometry of the face" led by Prof. LESLIE G. FARKAS (Toronto) between the 2nd and 4th of September.

On the occasion of the 1100th anniversary of the Hungarian Conquest the Department of Anthropology and the Móra Ferenc Museum, Szeged organized the conference titled "Hungarians of the Conquest period - Hungarians of the Arpadian-age" ("Honfoglaláskori magyarság - Árpád-kori magyarság") in Szeged from the 12th to the 14th of September, 1996. Lectures delivered during the Conference were published in a separate issue with the same title.

In connection with the Conference the exhibition titled "Bone Diseases of our Ancestors" (Elődeink csontbetegségei) was opened in the Móra Ferenc Museum, Szeged.



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